THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Master of Science (Tropical Agriculture)
DEGREE

Tropical Agriculture
FIELD

Interdisciplinary Graduate Program
PROGRAM

TITLE: Seed-borne and Transmission of *Bipolaris oryzae*, the Causal Pathogen of Brown Spot of Rice and Seed Treatment

NAME: Mr. Vu Van Ba

THIS THESIS HAS BEEN ACCEPTED BY

S. Sangchote **THESIS ADVISOR**
(Associate Professor Somsiri Sangchote, Ph.D.)

S. Prathuangwong **COMMITTEE MEMBER**
(Associate Professor Sutrudee Prathuangwong, Ph.D.)

S. Juntakool **COMMITTEE MEMBER**
(Assistant Professor Sunanta Juntakool, Ph.D.)

Somnuk Wongtong **PROGRAM CHAIRMAN**
(Associate Professor Somnuk Wongtong, Ph.D.)

APPROVED BY THE GRADUATE SCHOOL ON 21/12/2005

Vinai Artkongharn **DEAN**
(Associate Professor Vinai Artkongharn, M.A.)

THESIS

SEED-BORNE AND TRANSMISSION OF *Bipolaris oryzae*, THE CAUSAL PATHOGEN OF BROWN SPOT OF RICE AND SEED TREATMENT

VU VAN BA

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Master of Science (Tropical Agriculture)
Graduate School, Kasetsart University
2005

ISBN 974-16-1048-3

Vu Van Ba 2005: Seed-borne and Transmission of *Bipolaris oryzae*, the Causal Pathogen of Brown Spot of Rice and Seed Treatment. Master of Science (Tropical Agriculture), Major Field: Tropical Agriculture, Interdisciplinary Graduate Program. Thesis Advisor: Associate Professor Somsiri Sangchote, Ph.D. 70 pages.
ISBN 974-16-1048-3

Brown spot of rice, caused by *Bipolaris oryzae*, in paddy field was investigated for the relationship between disease severity on flag leaf and kernel infection at three growth stages including flowering, milky, and dough stage. Disease incidence and severity of brown spot were increased with stage of plant. There was a significant relationship between incidence of infected kernel and severity of infected flag leaf at 3 growth stages of rice plant; flowering ($r = 0.84$, $P < 0.0001$), milky ($r = 0.84$, $P < 0.0001$) and dough stage ($r = 0.80$, $P < 0.0001$). However, by artificial inoculation, the flowering and milky stage were susceptible to infection by *B. oryzae* than at dough stage.

Survival of *B. oryzae* in or on rice kernel was unchanged for 12 months at 10 ± 1°C whereas slightly reduced at room condition (20 – 41°C). Each part of infected kernel including embryo, endosperm, palea, lemma, rachilla, and sterile lemma was found infected by *B. oryzae*. Rachilla and sterile lemma were shown high level of infection at 82%, 79% respectively. Transmission studies of *B. oryzae* from infected kernel to seedling indicated that primary symptom was appeared on coleoptile and roots after 7 – 14 days. The first leaf of the seedling was also observed symptom after 3 – 4 weeks and some infected seedlings became browning and death in the later stage. Correlation coefficient between infected kernel and seedling infection showed highly positive ($r = 0.83$, $P < 0.0001$).

Rice kernel treated with fungicides including mancozeb (Dithane^{R}M-45), carbedazim (Bentox^R), mancozeb – carbedazim (Delsine^R Mx), and Carboxin – thiram (Vitavax^R 200). Carboxin-thiram was found the most effective and then followed by carbendzin-mancozeb, mancozeb, and carbendazim respectively. Infected kernel reduced from 12 % to 2.3, 2.8, 3.5, and 8% respectively in the seedling test. Dry heat treatment, at 70°C, and 80°C for 24, 48, 72, and 96 h of exposure showed significant reduction of *B. oryzae* in / on rice kernel. However, at 70°C for 96 h, and 80°C for 72 h of exposure found the most effective. The germinability was affected at higher temperature 90°C for 24 h and 80°C for 96 h of exposure to heat.

Student's signature

S. Sangchote
Thesis Advisor's signature

20/12/2005

ACKNOWLEDGEMENT

I wish to express my sincere appreciation and profound gratitude to my thesis advisor and chairperson of my advisory committee, Associate professor Dr. Somsiri Sangchote, for his invaluable guidance, wise counsel, constructive comments in improving the quality of this work and his assistance for preparing this manuscript as well as for the kind and sympathetic concern he showed for the author's personal and family welfare.

I am greatly indebted and heartfelt gratitude to the co-advisors of my advisory committee, Associate Professor Dr. Sutrudee Prathuangwong and Assistant Professor Dr. Sunanta Juntakool for their crucial advice, suggestions and kindness of reviewing the manuscript.

I would like to express my deep gratitude and thanks to Plant Genetics and Pathology Department, Vietnam Agricultural Science Institute for granting the study leave. I also extend my sincere appreciation to Sub Component 5, Seed Component, ASPS, Danida program for providing the scholarship and for their efforts to make contact with University authority and for the painstaking concern they showed for author's academic welfare.

To all of my friends, I extent my deepest gratitude for their continuous support, encouragement, optimism, constant moral, sharing happiness and difficulties during my study.

Finally, my special thanks and appreciation go to parents and my family, they have provided patience sacrifices understanding during stay far afield for this study and loving support in many ways. I could not have done without their.

Vu Van Ba

December 2005

TABLE OF CONTENTS

Page

TABLE OF CONTENTS (Cont'd)

Page

LIST OF TABLES

LIST OF TABLES (Cont'd)

Appendix Table **Page**

LIST OF TABLES (Cont'd)

Appendix Table **Page**

LIST OF FIGURES

LIST OF FIGURES (Continued)

Figure **Page**

LIST OF ABBREVIATION

ANOVA	=	Analysis of variance
a. i	=	Active ingredient
Car	=	Carbendazim
^{o}C	=	Degree Celsius
g	=	Gram
h	=	Hour
mm	=	Milimetter
Man	=	Mancozeb
MEA	=	Malt Extract Agar
NaOCl	=	Sodium hypochlorite
r	=	Correlation coefficient
RH	=	Relative Humidity
PDA	=	Potato Dextrose Agar
P	=	Probability
PSA	=	Peptone Sucrose Agar
Std	=	Standard Deviation
Std error	=	Standard Deviation of error
SAS	=	Statistical Analysis System
NUV	=	Near Ultraviolet

SEED-BORNE AND TRANSMISSION OF *Bipolaris oryzae*, THE CAUSAL PATHOGEN OF BROWN SPOT OF RICE AND SEED TREATMENT

INTRODUCTION

Rice (*Oryza sativa* L.) is the basal food of more than half the world's population, especially in Asia, Africa, Latin America and Near East and will continue to be their primary source of food in the future (Long Ping Yuan, 1995).

Rice kernel, like seeds of other crops, carries many organisms such as fungi, bacteria, nematode and other organisms. These seed borne organisms can be pathogens and saprophytes and more than 35 seed borne fungi have been recorded in rice (Richardson, 1979). One of the most important seed borne disease of rice is brown leaf spot, caused by *Bipolaris oryzae* (Breda de Haan).

Bipolaris oryzae (Breda de Haan) Shoemaker (syn. *Helminthosporium oryzae* Breda de Haan (syn. *Drechslera oryzae*. Br. De Haan), the anamorph of *Cochliobolus miyabeanus* (Ito and Kuribayashi) Drechsler), the causal agent of brown leaf spot disease of rice is a serious disease of rice worldwide. It causes losses in stand due to seedling blight, in yield due to leaf and culm infection, and in quality and yield by kernel infection. There were many reports about the damage disease such as Ocfemia (1922) recorded in Philippines in 1918. Padmanabhan *et al*. (1948) reported in Bengal, Aluko (1975) in Nigeria, Klomp (1977) in Surinam, and Datnoff *et al*. (1994) in Florida. Infected of rice kernel caused poor germination and could be a major source of inoculum for new crops raised from them, which caused losses (Rath, 1974). With seedborne infection, optimum control is often gained most cost effectively and safely, by the application of seed treatment, or through avoiding infection by producing seed in areas that are unfavorable for disease development, or using resistance varieties. These also achieved when applying to elimination *B. oryzae*. However, to gain more successive in these methods, knowledge on seed-borne

and transmission aspects was extremely important. Padmanabhan *et al*. (1953), Thomas (1940) and Ganguly (1946) reported infection of this pathogen from kernel to seedling. Besides, Suzuki (1930) studied the survival of *B. oryzae*. Datnoff *et al.* (1994), Mew *et al*. (1995), and Nyvall *et al*. (1995) reported location of *B. oryzae* inoculum on/ in the infected kernel.

According Neergaard (1977) establishment of a pathogen in/ on or with the seed – this implies that the pathogen is seed borne. Establishment and development of seedling infection was seed transmission. Seed transmission has been established if this completion of the infection course has been positively demonstrated to the exclusion of other means of transmission.

There are still many aspects needing for further investigation. Therefore, these experiments were carried out to studies some issues about seedborne disease of this pathogen.

Objectives

1. To determine the infection of *Bipolaris oryzae* at different stages of rice plant in the field and correlation between infections leaves and kernels
2. To determine the location of *Bipolaris oryzae* on/ in infected rice kernel
3. To study the transmission of *Bipolaris oryzae* from infected kernel infection to seedling
4. To investigate the survival of inoculum of *Bipolaris oryzae* on/ in rice kernel at different storage conditions
5. To control of *Bipolaris oryzae* in/ on rice kernel by seed treatment

LITERATURE REVIEW

Brown spot (also known as sesame spot, helminthosporiosis, seedling and leaf blight) of rice (*Bipolaris oryzae*) is widely and wide spread prevalent in the all the rice growing regions of the world (CMI Distribution Map 92), and the pathogen is seed borne (Feakin, 1970).

The disease is importance in many countries and has been reported as causing heavy losses when it reaches epidemic conditions. This disease was reported in the news when the Famine Enquiry Commission (1945) concluded that this disease was one of the principal causes of the famous Bengal famine of 1942 – 43. However, the disease is presented before that period, the description of the causal fungus was made by Breda de Haan in 1900. Ocfemia (1922) reported on the occurrence of the disease in the U.S.A, Japan, Italy, The Philippines and other countries. The disease is widely distributed throughout India (Rangaswami, 1996).

Nomenclature of the causal organism

Bipolaris oryzae (Breda de Haan) Shoemaker (syn. *Helminthosporium oryzae* Breda de Haan, the anamorph of *Cochliobolus miyabeanus* (Ito and Kuribayashi) Drechsler), the causal agent of brown leaf spot disease of rice, is the serious disease in rice production worldwide. *Bipolaris oryzae* belonging to Sub Division: *Deuteromycotina*, Order *Moniliales*, Family *Dematiaceae*.

Breda de Haan (1900), Hori (1901) and Hara (1916) described this leaf spot fungus and named *Helminthosporium oryzae* (cited from Ou, S.H 1985). Subramanian and Jain (1966) transferred *H. oryzae* to *Drechslera* as *D. oryzae*. Ito and Kuribayashi (1927) found the teleomorph of this fungus in culture and gave it the name *Ophiobolus miyabeanus*. Drechsler (1934) considered the fungus was belonging to the new genus *Cochliobolus* and Dastur (1942) formally transferred it to that genus. The name *Cochliobolus miyabeanus* (Ito and Kuribayashi) Drechsler ex Dastur is used at present (Ou, S.H 1985).

Chattopadhyay and Dasgupta (1959) showed that the conidia of *H. oryzae* germinated from one or more of intercalary cells or from both the end cells and named the fungus *Bipolaris oryzae*, as was done by Shoemaker (Padmanabhan, 1977).

Symptom of disease

Dickson (1956) described the brown cortical lesions appear on the coleoptile, subcrown internode, and seminal roots. Seedling blight occurred after emergence. Brown leaf spots and leaf sheath lesions developed on the less severely infected seedlings.

Mundkur and Chattopadhyay (1967) reported different types of symptom as dark brown, ellipsoidal to eye shape spots on the upper surface of the leaves. A fully developed spot has a grayish brown central region surrounded by a deep, reddish brown margin and was generally 4 – 6 mm. in size. Spot also appeared on leaves of older plants, on which they became larger than on the seedlings. On the coleoptiles, the spots were small brownish color. On the glumes, the disease appeared as dark brown or black oval spots when severely infection, the whole surface of the grain turned black and shriveled (Feakin, 1970). According to Padmanabhan (1977) reported that at the time of flower emergence typical brown, water soaked lesions appear at the node below the rachis or the neck. The fungus had been reported to cause spots on the grain and in severely attacked the affected grain shriveled. Ou (1985) also described symptom on the leaves were oval shape of sesame seeds. In addition, Datnoff (1994) described that brown spot symptoms initially appeared as small circular to oval spots on the first seedling leaves. Older spots on leaves had a bright yellow halo surrounding the lesion. Spots on the leaf sheath and hulls were similar to those on the leaves. Early brown sport lesions were difficult to distinguish from blast disease lesions.

The organism

Ito and Kuribayashi (1927) found an ascomycetous stage of *Helminthosporium oryzae* in culture, and gave it the name *Ophiobolus miyabeanus*. The perithecia were globes to depressed globes, and measured 560 – 950 x 368 – 377 µ. The asci were cylindrical or long fusiform, 21 – 36 x 142 – 235 µ, ascospore filamentous, pale olive green, coiled together, 6 – 15 septate, and measured 6 – 9 x 250 – 468 µ. However, Mew (2002) reported that the largest conidia might have 13 pseudosepta with a prominent hilum or basal scar. *B. oryzae* cultured on different media including PDA, PSA, and MEA and resulted in different sizes of conidia. The conidia have 5 – 9 septate, 39.56-101.89 x 11.96-16.10 µ on PDA medium, 4 – 11 septate, 43.47-101.43µ x 12.19-16.10 µ on the PSA medium, and 5 – 11 septate, 59.80-106.03 µ x 10.12-16.33 µ on the MEA medium.

According to Dickson (1956), *B. oryzae* produced grayish – brown to dark – brown mycelial mats in/ on the plant parts, and in culture. The conidiophores form in mats or singly, light brown to olivaceous and vary greatly in both width and length. The conidia were brown, slightly curved, tapering toward the round apex and toward the base, and vary in shape and septation. In addition, Padmanabhan (1977) also reported the conidiophores emerged single, generally 7 – 14 septate, swollen basal cell, and with geniculations at the apical end. The conidia were curved, sometimes straight or curved to one side. Mew (2002) found two types of fungal detection on rice kernel: type I produced less conidia and abundant aerial mycelia, gray, greenish gray to black; conidiophores were slender, and conidia were darker than mycelia. Meanwhile, type II produces abundant conidia and aerial mycelia were either absent or scanty. Conidiophores were straight or flexuous, brown to dark brown and bearing 3-5 conidia at the end and on the sides. About physiological characteristic of *B. oryzae*, Ou (1985) cited from Nisikado (1923, 1926) that the fungus grew over a wide temperature range, the optimum temperature for mycelial growth was 27 – 30^{o}C(at pH 6.6 – 7.4), and conidia germinated 25 – 30^{o}C (at pH 2.6 – 10.9). The conidia were formed between 5^{o}C to 38^{o}C (at pH 4 - 10). Cited from Ocfemia (1924) that the

optimum temperature was 28^{o} C, minimum 16^{o}C, and maximum 40^{o}C for mycelial growth.

Yield losses

Brown spot disease has received more attention in recent years, damage is frequently high. Three phases of damage result from the disease including losses in stand due to seedling blight, in yield due to leaf and culm infection, and in quality and yield by kernel infection. Infected kernels germinate poorly, with the result that a certain proportion of the kernel is loosed. Ocfemia (1922) recorded the mortality was 10 to 50 per cent of seedling of susceptible varieties in Philippines in 1918. It was commonly found in seed beds in which every seedling were heavily infection. Tucker (1927) recorded 15 per cent of death seedlings in varietal trials in Puerto Rico. In the grain infection phase, little was known about the losses. Padmanabhan *et al*. (1948) mentioned that when an epiphytotic breaks out, almost a total destruction of the crop might be expected. Losses in yield up to 90 per cent had been recorded during the epiphytotic in Bengal. In the Bunjab, losses in weight of grains ranging from 4.6 to 29 per cent had been reported (Bedi and Gill, 1960). In Japan, disease was regarded the annual reduction in yield loss had been estimated at 23 - 28,000 tons (Kawada *et al*., 1954).

In Nakhon Nayok, Prachin Buri, and Thon Buri in Thailand, seedling blight was common in seedbeds. In the field, destruction of leaf reduced leaf area and yields. Many experimental rice plants infected by brown spot at the flowering stage with loss of grain weight about 22 percent (Worawisitthumrong *et al*., 1971).

Bedi and Gill (1960) estimated that *H. oryzae* caused 4.58 to 29.1 per cent loss in weight of rice grains and 11.0 to 37.3 per cent reduction in germination (Padmanabhan, 1977). In Nigeria, Aluko (1975) estimated that severe infection reduced the yield by 30 to 43 per cent. Klomp (1977) claimed that the disease caused early senescence of rice in Surinam and if prevented, up to 50 per cent increased in yield might be expected (OU, 1985). Datnoff *et al*. (1994) reported that the disease

could adversely affect the yield and milling quality of the grain. Under environmental condition conductive to disease, yield loss estimates range from 16 to 40 percent in Florida.

Inoculum location in/ on the kernel

Suzuki (1930) found the fungus was not only within discolored kernels but also internally in kernels apparently healthy from external appearances. Discolored kernels, on planting, gave rise to infected seedlings. According to Thomas (1940), Ganguly (1946) primary infection through diseased kernel was probably most common, although diseased kernels did not necessarily always gave rise to infected seedlings. Thomas (1941) also reported that *Helminthosporium* disease was soil borne and seed borne. Infection could take place from soil and it was possible that conidia could survive in soil from one crop to the next. In addition, Nisikado and Nakayama (1943) found mycelia only in the pericarp and seed coat. Besides, according to Ganguly (1946) the conidia of the fungus were air borne and presented in the air throughout the off season, and infected early in the seed beds. Chattopadhyay (1952) stated that primary infection related to the presence of the pathogen in the kernel whereas the disease in subsequent stages was result of infection by air borne conidia. Studies on the viability of the fungus in natural condition Padmanabhan *et al*. (1953) showed that the viability was remained only in kernel. Primary infection was owing to the presence of the pathogen in the kernel. Meanwhile, Chattopadhyaya and Chakraborty (1954) found that the fungus did not survive in the soil, but did so only in the stubble. Imam Fazli and Schroeder (1966) reported that infection of the embryo occurred under favorable condition and the mycelia were reported remaining in endosperm and grew along the cell walls of endosperm. Bernaux (1981) found *D. oryzae* could survive on and be transmitted by kernels. The palea and lemma were susceptible that was determined by inoculation but could survive for short period. The earlier infections, mycelium and conidia remained between the glumes and caryopsis. According to Suzuki (1985), diseased hulls were numerous in empty glumes and pedicels than lemma and palea. In grains, the hilum and placenta were severely infected than other areas. Moreover, Luh (1991) reported that the infected kernel was

likely to be the carrier of the pathogen and the primary source of inoculums. Air borne conidia were responsible for secondary infection as well as the spread of the disease to adjacent fields. Mew and Gonzales (2002) mentioned that *Bipolaris oryzae* was often observed on the entire kernel surface (about 32%) or on sterile lemmas (29%). Suparyono *et al.* (2003) reported that infected kernels, volunteer rice, infected rice debris, and several weeds were the major sources of inoculum in the field. Khokon *et al*. (2005) studied on the presence of fungi in inert matter of rice kernel showed that the kernel lot with inert matter (60 %) including broken kernels, husk and awn, *B. oryzae* was detected.

Survival and longevity

In Japan, Kuribayashi (1929) survival of conidia on infected kernel varied from 396 to 859 days. Mycelium in infected tissue was viable from 1044 to 1076 days. According to Suzuki (1930) who found that the fungus could survive in the kernel for four years at least. Nisikado *et al*. (1938) found that the fungus remained viable from 28 to 29 months at 30^{o}C, but was unable to survive at 35^{o}C for more than 5 months. The fungus was seed borne, and survived from one crop to next on infected rice kernel straw and stubble. Thomas (1940) found that conidia of *B. oryzae* survived in the soil from one crop to next crop. Besides, Page *at al*. (1947) found that the conidia retained their viability at low temperature (2^{o}C) with high relative humidity (81%) after 100 days. They also found that the variability of the conidia was slightly reduced after storage for six mouths at 31^{o}C and 20%. R.H. At the same temperature and 95 % R.H, conidia failed to germinate after one month. At 45 %. R.H, many conidia survived six months. Under warm and humid conditions, the conidia would not survive very long and the fungus commonly stayed over winter in infected plant parts. Meanwhile, Lam (1973) stated that the hyphal fragments of *C. miyabeanus* remained viable for at least 6 months at 20^{o}C and 31 % RH.

Padmanabhan *et al*. (1953) studies the viability of *H. oryzae* in kernels, leaves, nodes, internodes, and stubble left in the field after harvest and in the soil during December to July. He found that the pathogen remained viable only in the kernel during this period. On the contrary, Chattopadhyay and Chkrabarti (1954) found that

the fungus survived in the stubbles till June, but not in the soil. Dickson (1956) reported that the fungus persisted over unfavorable periods in both conidial and mycelial phases and might live for two to three years in infected plant parts and infected kernels. Ghose *et al*. (1960) had found conidia presented in the air near rice fields throughout the off-season. Rungaswami and Ramalingam (1962) reported that *B. oryzae* survived for short periods when added to unsterile soil, either as conidial suspension or infected kernels than when added to the steriled soil. From infected kernel added to unsteriled soil, several antagonistic micro-organinms were isolated.

Chandwani *et al*. (1963) showed that the conidia of *H. oryzae* occurred over rice fields from December to July, as sources of primary seedling infection. He also commonly found the conidia and mycelium on crop residue. According to Worawisitthumrong *et al*. (1971), the viable conidia remained on rice stubble in the field and on husks of grain in storage. They trapped conidia from June to December at other locations for several years; conidia were presented at every month. Dhanapal (1979) studied on the survival of *Helminthosporium oryzae* in soil and reported that conidia of *H. oryzae* could survive in the sterile soil up to 130 days, but in unsterile soil was 85, 115 days, respectively. Kulkarni (1981) reported that *Drechslera oryzae* was unable to survive for up to 8 weeks in semidry and up to 4 weeks in puddled unsterilized soil and up to 6 and 9 weeks respectively in sterilized soils. Besides, Ganesan (1993) reported that conidia of *Drechslera oryzae* were mixed with loam soil that had been collected from rice field, and stored at a moisture content of 60 % for one year and then dried. After 6 years, 2 % the conidia were still viable. Datnoff *et al*. (1994) reported that *Bipolaris oryzae* could also survive on infected rice straw and stubble. Wesely (1996) found that *Cochliobolus miiyabeanus* survived for up to 120 days after inoculation at all stages of grain development and could survive up to 150 days after inoculation when inoculated at the flowering stage. Mia *et al*. (2000) studies on survival of seed borne inoculum of *B. oryzae* and reported that inoculum of *B. oryzae* was not affected at or below 82.9 % RH throughout the stored period of 10 months. Pathogen lost their viability at 100 and 92.9 % RH by 5 and 9 months. However, at or above 75.6 % RH, external borne conidia could not survive over 135 days. The conidia remained viable after 165 days of storage at 26.8 to 66.8 % RH was

18 – 42 %. They also showed that under room temperature conditions only 1 % of conidia survived at 105 days and none survived beyond this period. Meanwhile, the incidence of internal inoculum of pathogen in kernel remained for 300 days at 25 ± 1^{o}C, and viability of pathogen gradually declined when kernel with high moisture content (16%) at 25 ± 1^{o}C. Suparyono *et al*. (2003) reported that the fungi survived in the kernel for more than 4 years.

Transmission and infection of *Bipolaris oryzae*

Conidia of *H. oryzae* germinated by extrusion of a germ tube from the tips of the apical and basal cells where the cell walls were the thinnest. The germ tubes were surrounded by thick, mucilagious sheaths which enable them to adhere to a solid surface. After a few hours, the tips swept to form branched appressoria and then formed peg like infection hyphae which penetrated directly through the cuticle and epidermis (Nisikato and Miyake, 1922). Ocfemia (1924) observed the first symptoms of infection within 24 hours of inoculation. From a single leaf spot under flavor humid conditions, conidia and conidiophores were produced, dispersed and landed on adjacent part of other leaves and cause new infection. Nisikato (1923) found the conidia germinated at optimal temperature between 25 to 30^{o}C, the maximum at 41^{o}C and minimum at 2^{o}C. The optimal temperature for mycelium growth was between 27 ^{o}C to 30^{o}C. Conidia were formed between 5 and 35^{o}C. Besides, Katsura (1937) found that germination of conidia was at 25^{o}C and relative humidity of 92 % or over. Tucker (1923) found that a *Helminthosporium* caused infection of the roots of seedlings and Hemmi and Yokogi (1928) confirmed this by artificial inoculation under several conditions. The infection carried in the kernel caused the first occurrence of the disease, the young seedling showed signs of infection soon after germination (Suzuki, 1930). Moreover, Ganguly (1946) reported that the coleoptile and sometimes roots were often infected from diseased kernels and during seed germination, necroses were found on coleoptiles and sheaths of the first leaves (Bernaux, 1981). Ito (1932) reported that infection of the leaf sheaths and of the glumes took place in a similar manner to infection of leaves on the lesion. Within three to four days after infection had taken place, lesion appeared. According to Sato (1965), the susceptibility of the

rice plant to the disease increased with age. At the early stage of the development of the rice plant, only minute spots were formed and after ear formation, large spots developed on the lower leaves. Akai *et al*. (1965) reported that the number and size of spots developed were positively correlated with the concentration of conidia in the suspension.

Imam Fazli and Schroeder (1966) found that the rice kernel was more susceptible at flowering and milky stage than at the soft dough or mature stage, and Duraiswamy *et al*. (1983) found the same results. Meanwhile, Marchetii and Petersen (1984) found no correlation between brown spot symptoms and floral abortion, but kernel discoloration was moderated with brown spot. Mondal *et al*. (1998) also obtained the positive correlation between the incidence of leaf spot and grain spot in their study. Studied on development of fungal brown spot on cultivated wild rice in Minnesora, Nyval and Percich (1999) reported that the percentage of *B. oryzae* of all lesions increased from early grain formation until maturity. About kernel infection, Watanabe *et al*. (1976) reported that the most destructive injury to hulls followed hyphal invasion of parenchymatous tissue through the inner epidermis of the hulls at flowering. The germ tube passed through the space between the lemma and palea to reach the inner epidermis. The outer epidermis of the hull was protected from the fungus by a silicate layer which increased at maturity. Invasion occurred through the basal part of the trichomes and the joints of the outer epidermal cells.

Zad and Khosran (2000) studied on infection of *B. oryzae* on several rice cultivar seeds by using blotter method, the abnormal seedling rates caused by this pathogen was second (31.4%) after *Alternaria padwickii* (33%). Mia *et al*. (2001) showed effect of seedborne *B. oryzae* on seed germination and disease development in the field. Rice kernel samples exhibiting 70 % natural infection of *B. oryzae* mixed with healthy kernels to produce 0, 7 17.5, 35, and 70 % infection levels. Reduction in the emergence of seedlings under different infection levels ranged from 7.2 to 30.4, 13.0 to 40.5, and 9.1 to 47.4 %, and there was no relationship between initial kernel infection and subsequent kernel infection. In addition, the effect of different incidence level of *B. oryzae* in rice kernels on kernel-seedling transmission was also mentioned

by Malavolta *et al*. (2002) in their study that significant correlation coefficients were observed between kernel infection and seed germination (r = -0.89), kernel infection and dead or infected seedling (r = 0.66). The similar result also obtained by Imolehin (1983) and Kulik(1977). Suparyono *et al*. (2003) reported that infected kernels gave rise to infected seedlings, and the fungus spreaded from plant to plant and in the field by airborne conidia.

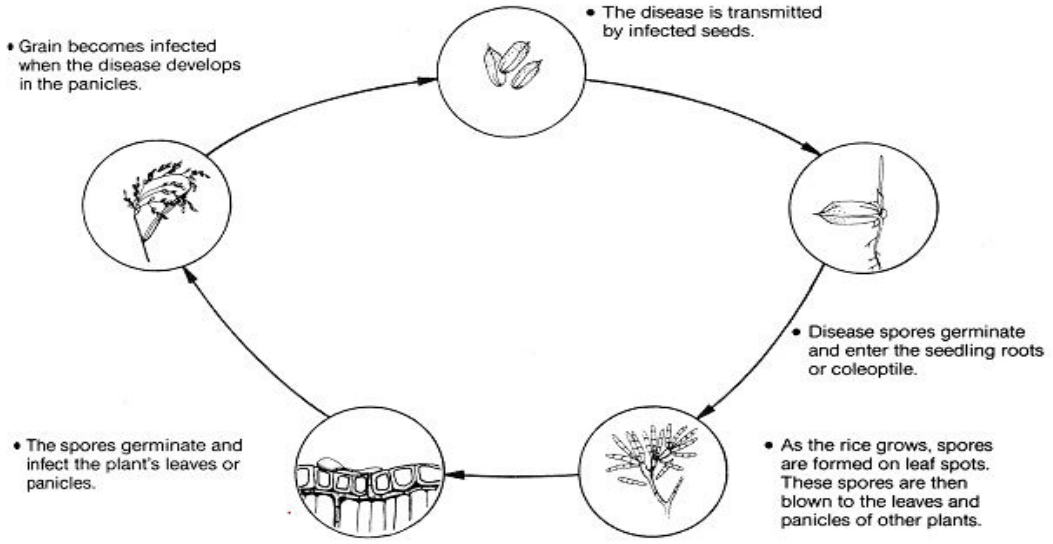


Figure 1. Life cycle of *Bipolaris oryzae*, causal brown spot of rice.

The conidia were carried on or hypha packed in the rice kernel (Imam Fazli, 1966). When the condition was favorites, conidia germinated and entered the seedling roots or coleoptile. As the rice plant grew, conidia were formed on leaf spots. After that the conidia were blown to the leaves and the panicles of the other plants. The conidia germinated and infected the plant's leaves or panicles. Finally, the grain became infection when disease developed in the panicle, crop yields were drastically reduced (Suparyono *et al*., 2003).

Control of *Bipolaris oryzae* through seed treatment

Control of this disease is being attempted by many measures such as breeding resistant varieties, treating the kernels with fungicides and hot water, spraying, and adjustment of agronomic practices etc.

Hot water treatment: Chaudhuri (1948) reported that the pathogen could be controlled by immersing kernel for 10 – 12 minutes in hot water at 53 – 54^{o}C before sowing. According to Mallikarjuna Rao (unpublished), presoaking in cold water for 8 hrs and followed by hot water treatment at 52^{o}C for 15 – 20 minutes was effective in controlling seedborne infection in most varieties. A reduction of infection from 100 to 20 per cent was obtained by immersion of diseased kernels in hot water at 55^{o}C for 10 minutes Thomas (1941). Suzuki (1930), and Reyes (1939) also used hot water to treat rice kernel to Helminthosporiose and also resulted reduces of infection. Kulkarni (1980) pre-soaked infected kernel of rice in cold water and then treated with hot water at 52^{o}C for 10 min and showed no significant different in degree of infection. Krishnamurthy (2001) treated rice kernel sample infected by *Drechslera oryzae* in hot water (at 50, 55, and 60^{o}C for 10 minutes) and the result showed that hot water treatment at 60^{o}C was the most effective.

Fungicidal treatment of seed: Some fungicides used at the Central Rice Research Institute such as Arasan, Phygon, Cuprocide, Agrosan GN, had no effect on germination, disease development and yield in rice varieties (Ghose and *et al*., 1960). Nisikado and Miyake (1922) reported that dressing with mercuric chloride, silver nitrate, copper sulphate, calcium hypochloride, formaldehyde, and phenol was effective, giving partially control.

Khatua (1978) used 9 systemic and 3 contact fungicides to treat rice kernel against *Cochliobolus miyabeanus* and reported that Dithane M-45 (mancozeb), Vitavax (Carboxin) and Sicarol (Pyracarbolid) were the most effective. Vihyasekaran (1980) mixed the fungicides guazatine and dichloromethan to eradicate *Drechslera oryzae* from all parts of the kernels when immersed in the mixture for 1 h. Kannaiyan

(1982) used potassium chloride and diamonium phosphate resulted in reductions in seedling infection by *Helminthosporium oryzae*. Valarini (1988) found that the effective treatments were guazatine - imazalil, iprodione, and iprodione + thiram to control *Helminthosporium oryzae* when treated rice kernel with highly infection of this fungus by thiram, captan, guazatine - imazalil, iprodione, iprodione + thiram and imazalil.

Recently, Sachan (1994) treated *Helminthosporium oryzae* infected rice kernel with captan, Ceresan [phenylmercury acetate], Dithane M-45 [mancozeb], thiram, Bavistin [carbedazim], Bavistin - Dithane M-45(1:1) and Bavistin - thiram (1:1). Bavistin - Dithan M-45 and Bavistin - thiram were more effective than others. Besides, Buffa (1995) treated rice kernel sample with high level of infection of *Cochliobolus miyabeanus* with these fungicides: Rovral (iprodione 50%), Rovral FL (iprodione 25 %), Iprovax Flo (carboxin - iprodione 24% - 18 %) Vitavax Flo (carboxin - thiram 17.21% + 17.21%) and Tridex FL (mancozeb 38 %) and iprodone almost eradicated *Cochliobolus miyabeanus* and Vitavax Flo showed a phytotoxic effect at double doses. According to Rangaswami (1996), soaking the kernel for 48 hr in 1,000 dilution of Uspulan, an organic mercurial, was effective in eradicating the externally seed borne pathogen. Seed treatment with captan, thiram, chitosan, carbendazim, or mancozeb has also been found to reduce seedling infection.

Krishnamurthy (2001) also used fungicides carbendazim (Bavistin), and benomyl (Benlate) at 0.1, 0.2 and 0.3 g/ 100g seed and benomyl at 0.1 % reduced the incidence of infection of *Bipolaris oryzae*. Moreover, Parisi (2001) also applied following fungicides (in grams of active ingredients per 100 kg of seeds): prochloraz-carbendazim (16.2-60.0), prochloraz-carbendazim (21.6-80.0), prochloraz+carbendazim (27.0-100.0), carbendazim+thiram (30.0-70.0), carbendazim-thiram (37.5-87.5), carbendazim-thiram (45.0-105.0), prochloraz-fluquinconazole (20.4-100.2), carboxin+thiram (60.0-60.0) and pyroquilon (400.0) to infected kernel and resulted significantly reduced the incidence of infection. Suparyono *et al*, (2003) also found that seed treatment with tricyclazole gave a good control of *B. oryzae*.

MATERIALS, METHODS, AND RESULTS

1. Infection of *B. oryzae* at different stages and relation of disease severity at different growth stages of rice

The growth stage of the host and some other factors such as the time of infection, weather at the infection time as well as intrinsic organism relate to degree of infection of organism on/ in the seed. Depending on each growth stage of host plant which decide the success of infection of organism and amount of infection. In addition, the rate of increase of inoculum also relate to the extent of the period from infection to sporulation of the parasite in the host (Neergaard, 1977). In these experiments, the different growth stages of rice plant will be studied their effect on an infection and disease development of *B. oryzae.*

1.1. Materials and methods

1.1.1. Disease severity at different growth stages of rice

Flag leaf of individual tiller and its panicles were collected at the flowering, milky, and dough stages from experimental fields (Supanburi cultivar), Kasetsart University, Kampaengsaen campus in rainy season 2003. With three replications, each replication 15 tillers were collected and the flag leaves were dried by arranging separately on newspapers or filter paper. Infected leaf area was measured using the method as described by Lamaban (2003): Leaves of *B. oryzae* infected plants were detached and placed on a sheet of white paper. A tracing paper was placed over the leaves to draw the outline of the entire leaf margin and infected portion of leaves. The total leaf area (mm^2) and infected leaf area (mm^2) were recorded and calculated to infected area (%). Correlation between severity of the infected flag leaf and incidence of infected kernel was estimated. The panicles after counting number of infected kernels were also dried and stored in the low temperature incubator at 10^oC. Additionally, the kernels and leaves with spot symptom will be further investigated and examinated for an infection of *B. oryzae* by blotter method before severity and

incidence were calculated. Infected leaf area and incidence of infected kernel (%) were also investigated.

$$\text{Infected seed (\%)} = \frac{\text{Infected seed of panicle}}{\text{Total seed of panicle}} \times 100$$

$$\text{Infected leaf area (\%)} = \frac{\text{Infected leaf area of flag leaf}}{\text{Total leaf area of flag leaf}} \times 100$$

1.1.2. Infection of *B. oryzae* at different stages

The experiment was conducted in the greenhouse, using a completely randomized design with three treatments, and five replications. Rice cultivar Suphan Buri used in this experiment. Rice kernels were incubated in the moistened towel for 3 days at room temperature. After germination, seedlings were transferred to the pots using 2 seedlings per pot. These seedlings were sown at 7 days interval. Therefore, at the inoculation time, three studied stages of rice plant including flowering, milky, and dough stage were obtained.

The single conidia were isolated from infected kernel after incubating on moistened blotter paper for 7 days, cultured and maintained on the PDA medium with rice leaf extract. The conidia were collected and suspended in distilled water to the final concentration of 5 x 10^4 conidia/ ml by using hæmocytometer. The suspension was uniformly mixed and sprayed using air burst for inoculation to the plants. The control treatment was also done by the same way, using distilled water instead of spore suspension. After inoculation, inoculated plants were kept at high relative humidity. 15 days after inoculation, the panicle and flag leaf were collected and checked for infected kernel and infected leaf area (as previous experiment). The infected kernel and typical symptom were confirmed by using blotter method before calculating results.

1.2. Statistical analysis

Data was analyzed an analysis of variance (ANOVA) and mean comparison by SAS version 6.12 (SAS Institute Inc). Statistics graphics and correlation coefficients was used Sigma Plot 2000 program, version 6.0 (1986 – 2000 SPPS Inc).

1.3. Results and Discussion

1.3.1. Disease severity at different growth stages of rice

In the experiment fields, the different of infected levels on flag leaf and kernel of whole panicle of infected plant was observed at three stages: flowering, milky, and dough stage. The paddy field which almost at dough stage, was severely infected by *B. oryzae* and produced brown spot on the flag leaves and discoloration on the kernel of the panicle whereas at the flowering and milky stage were slightly. The results showed the incidence and severity of brown spot were increased according to the development stages of plant from flowering till dough stage (Figure 5). The mean of incidence of infected kernel was 26.01% at the dough stage, and severity of infected flag leaf was 1.6%. Meanwhile, the incidence of infected kernel was 15.1%, 12.4 %, and severity of infected flag leaf was 0.6%, 0.4% at the milky and flowering stage respectively. The incidence of infected kernel was significantly correlated with severity of infected flag leaf at each stage (Figure 2, 3, 4). The percentage of infected kernel showed a significant correlation ($r = 0.80$, $P < 0.0001$) of severity of infected flag leaves at the dough stage. The milky and flowering stage also showed correlation at $r = 0.84$, $P < 0.0001$; and $r = 0.84$, $P < 0.0001$, respectively.

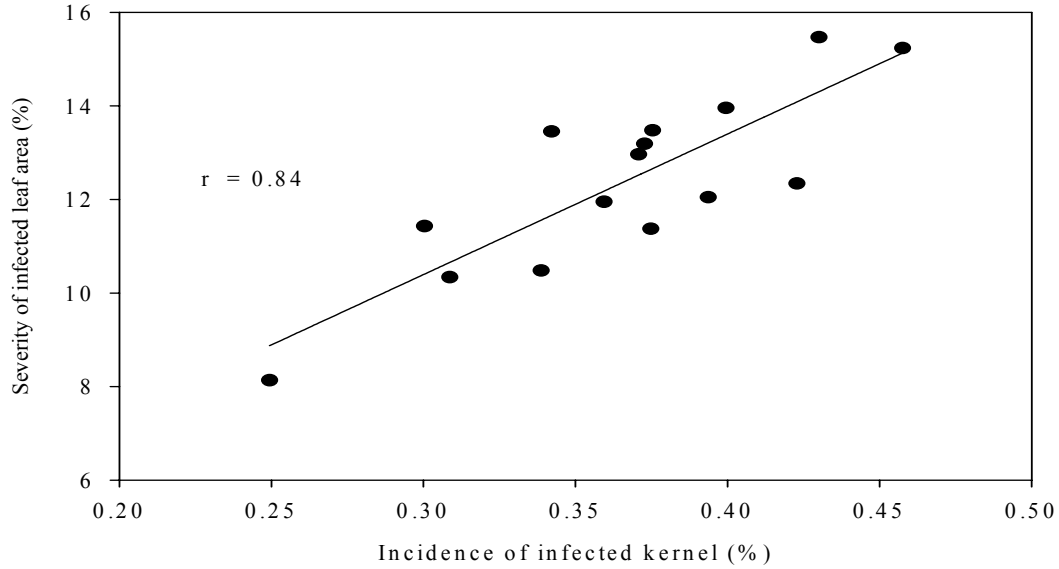


Figure 2 Relationship between severity of brown spot on flag leaf and incidence of *B. oryzae* on the kernel at the flowering stage (r = 0.84, P < 0.0001) in the field.

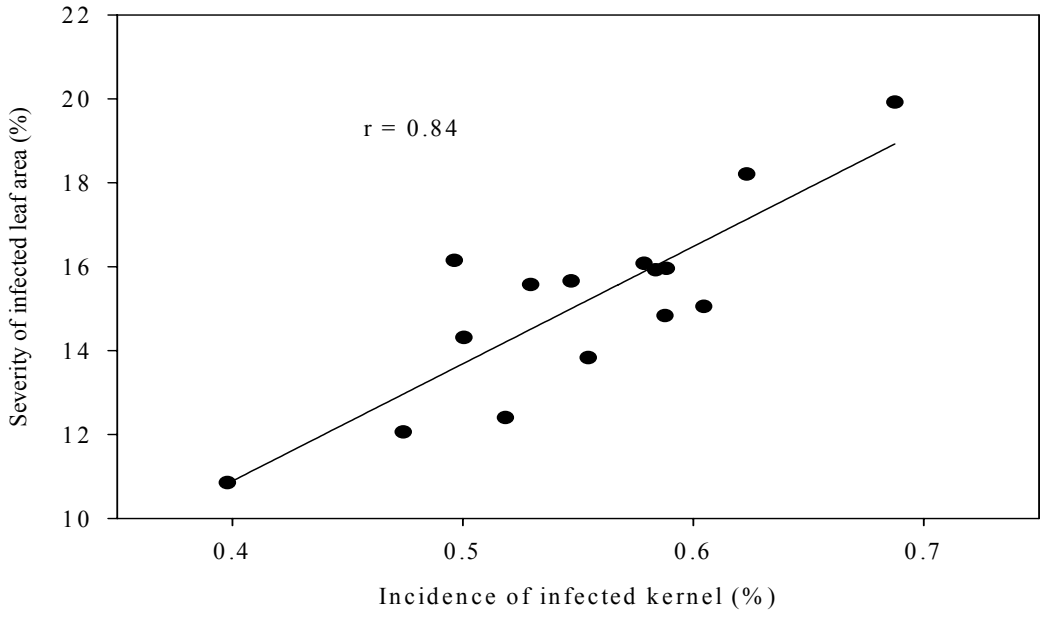


Figure 3 Relationship between severity of brown spot on flag leaf and incidence of *B. oryzae* on the kernel at the milky stage (r = 0.84, P < 0.0001) in the field.

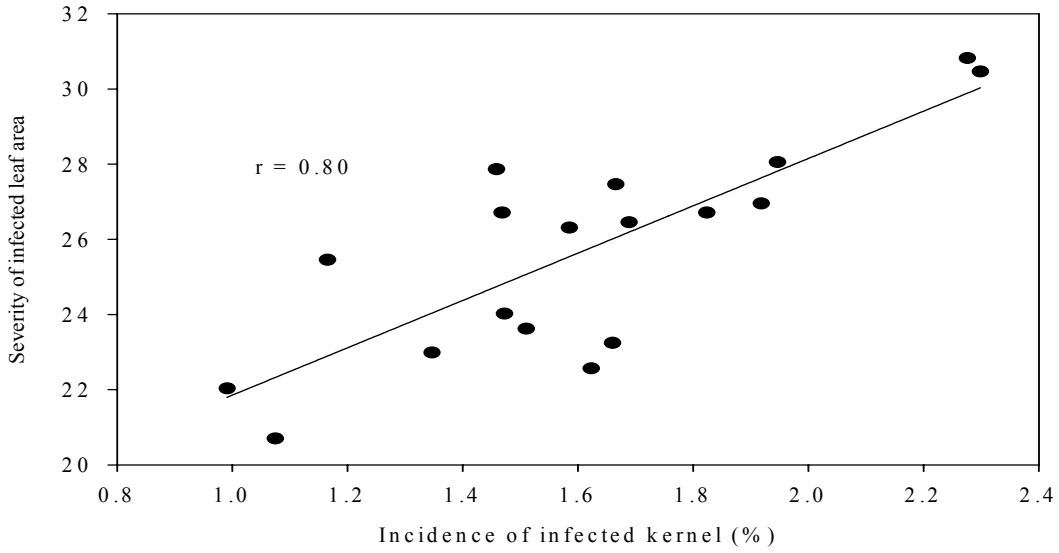


Figure 4 Relationship between severity of brown spot on flag leaf and incidence of *B. oryzae* on the kernel at the dough stage (r = 0.80, P < 0.0001) in the field.

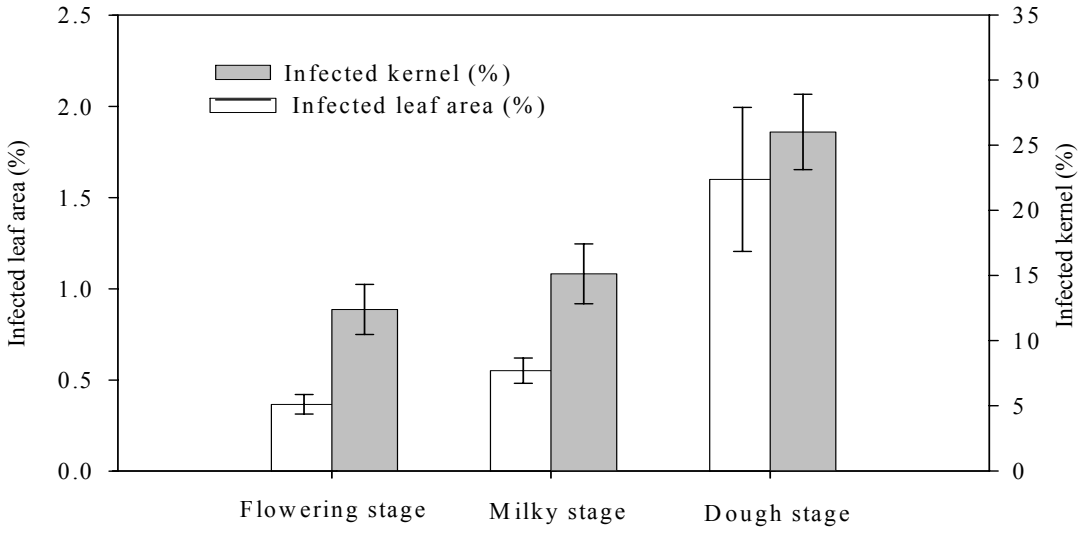


Figure 5 Incidence of infected kernel (%) and severity of brown spot (%) at flowering, milky, and dough stage of rice plant in the field.

1.3.2. Infection of *B. oryzae* at different stages

Three stages of rice plant growth including flowering, milky, and dough presented at the inoculation time. 5-6 days after inoculation, the disease symptoms occurred with a small spot that could be visible by naked eyes both on leaves and glumes. 15 days after inoculation, the panicles and flag leaves were collected from all treatments. Then, the infected kernel and total number kernel per each panicle were counted. Besides, the flags leaves were dried in the herbarium, then, the number spots on leaf were counted, total leaf areas also were measured. Finally, the percentage of infected leaf and percentage of infected kernel were calculated and analyzed for results.

The relationship between infected leaf area and infected kernel were measured by using correlation coefficient for each stage flowering, milky, and dough. The results showed that there were positive and moderately correlations between them in which at the flowering stage $r = 0.54$, $p < 0.001$(Figure 6), the milky stage $r = 0.52$, $p < 0.001$(Figure 7), and the dough stage $r = 0.50$, $p < 0.001$(Figure 8) respectively. The infection of *B. oryzae* found both inoculation and non inoculation (control). However, there was highly significant different between inoculation and non inoculation treatment. In the inoculation treatment, the mean of percentage of infected kernel and percentage of infected leaf area were also compared among these stages and the results showed in the figure 9. The highest percentage of infected leaf area (1.21%) was resulted at the flowering stage and milky stage but not significant and the lowest percentage of infected leaf area (0.76%) was at the dough stage. Similarly, percentages of infected kernel were significant difference among these stages. At the flowering stage was the highest infection of *B. oryzae* at 16.66% followed by milky stage at 11.81%, and the lowest infection at 6.62% at dough stage. Meanwhile, in non inoculation, the results indicated that the infection was able to be infected from other sources in which the infected kernel was 0.2 and 0.05 percentage of infected leaf area. Therefore, the most susceptible growth stage of rice plant was at the flowering followed by milky, and lastly dough stage. The observation of infected seed of these stages showed that the infection at the flowering stage caused an empty seed

compared with milky and dough stage. Some glumes became black, and could not develop further. Thus, the infection in the early stage of seed development was the major causes of loss in yield (Imam Fazli *et al.*, 1966).

These results were similar to Padmanabhan and Ganguly (1954), they also found the most susceptible to *B. oryzae* was flowering stage, and Imam Fazli *et al*. (1966) also found that the flowering and milky stage was the most susceptible to the pathogen. These results were contrasted with the results which obtained from the rice field. However, according to Fukatsu and Kakizaki (1955) found that the conidia to be initially produced on the old lesion, and Sato (1965) found the formation of conidia on the various types of spots and under favorable conditions aerial mycelium grew out from the lesions and formed secondary conidia that infected to other lesions and leaves. Moreover, Padmanabhan *et al*. (1954) also reported that at the later stage, the spots developed larger than early ones. An increasing in percentage of discolored seed was contributed to the spread of the fungus from loci of primary infection to adjacent seeds during the continued development of the plant (Imam Fazli *et al.*, 1966). They also reported that the progressive decrease in percentage of discolored seeds and spots on leaf associated with increasing maturity at the time of inoculation indicated either increased resistance related with increased maturity or the effect of time limitations for infection to develop or spread from primary infection loci. Consequently, these could be concluded that the infection was the most susceptible at the flowering stage, and then, the infection continuously developed at the later stages under favorable condition on both leaves and seeds.

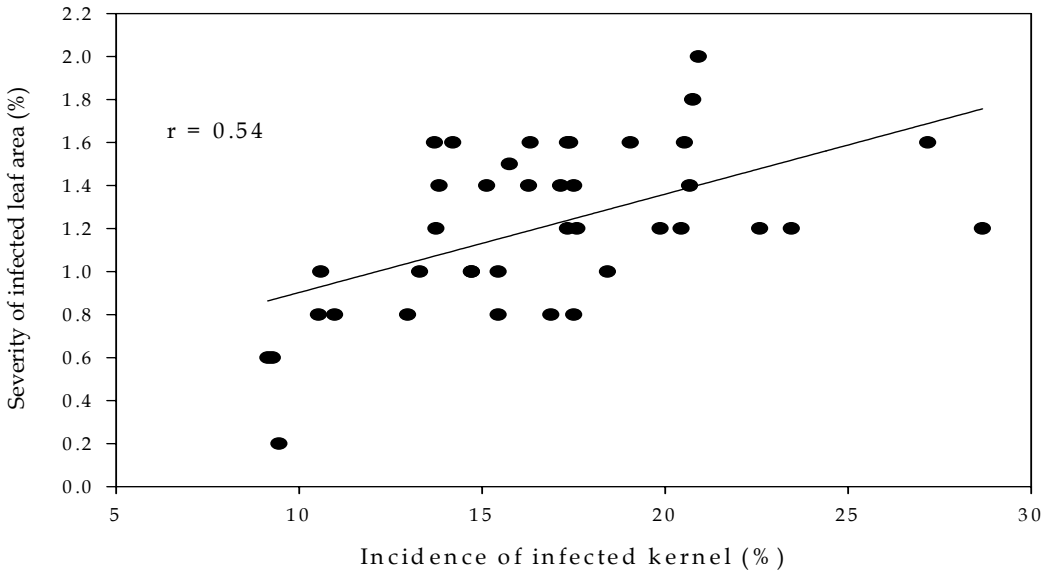


Figure 6 Relationship between severity of brown spot on flag leaf and incidence of *B. oryzae* on the kernel at the flowering stage (r = 0.54, P < 0.0001) by artificial inoculation in the greenhouse.

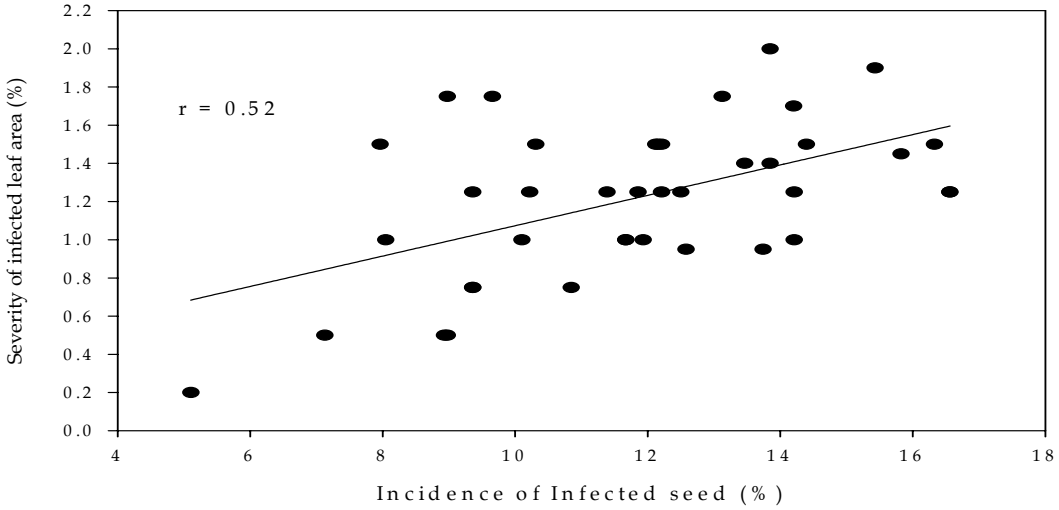


Figure 7 Relationship between severity of brown spot on flag leaf and incidence of *B. oryzae* on the kernel at the milky stage (r = 0.52, P < 0.0001) by artificial inoculation in the greenhouse.

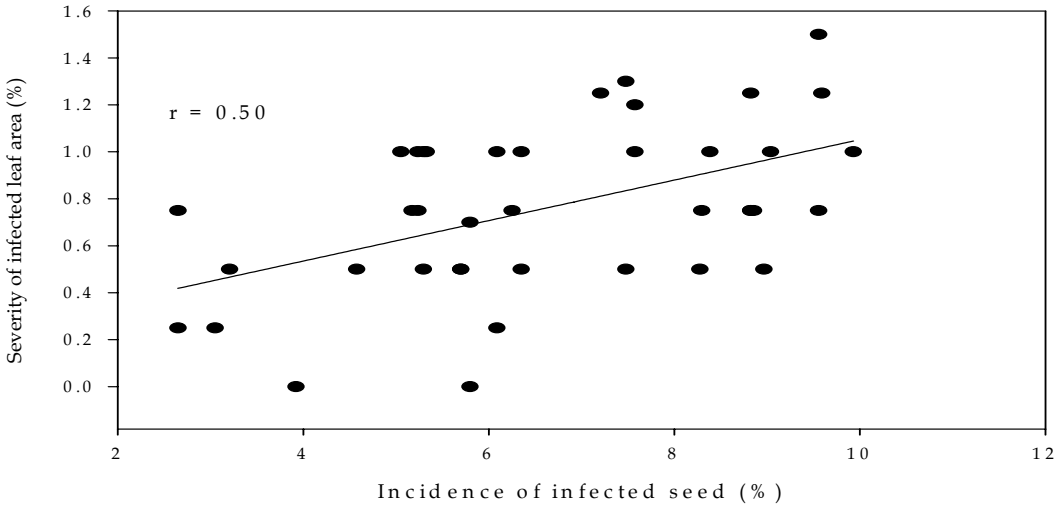


Figure 8 Relationship between severity of brown spot on flag leaf and incidence of *B. oryzae* on the kernel at the dough stage (r = 0.50, P < 0.0001) by artificial inoculation in the greenhouse.

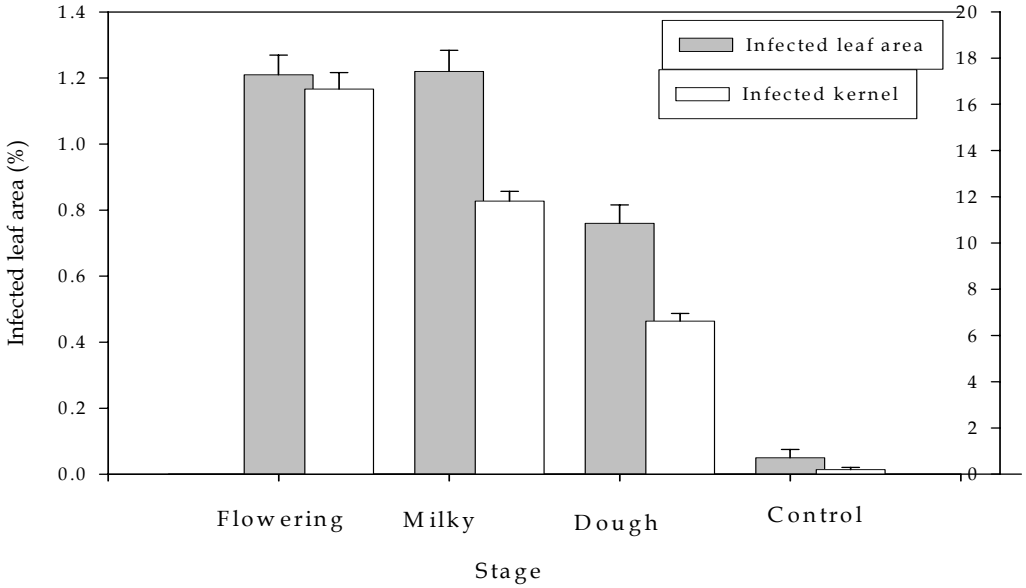


Figure 9 Incidence of infected kernel (%) and severity of brown spot (%) at flowering, milky, and dough stage of rice plant by artificial inoculation in the greenhouse.

2. Location of *Bipolaris oryzae* on/ in rice kernel

The majorities of seed borne pathogens are carried passively on seed surfaces or is established as hyphae or conidia in or under the seed coat. Successful transmission to developing seedlings often depends on the location and the mount of inoculum presenting on or in the seed. There are some techniques that have been used to determine inoculum of pathogen in seed, including whole mount preparation, seed component plating, microtome sectioning, and embryo counting (Maude, 1996). The objective of this experiment was to study the location of *B. oryzae* in/ on rice seed by using seed component plating technique.

2.1. Materials and methods

Kernel samples of rice infected with *Bipolaris oryzae* were collected from experimental field (Supanpuri variety), Kasetsart University, Kampaengsaen Campus and then stored at 10^{o}C before studying. The infected kernels were selected from seed lot and tested using the component plating method as described by Neergaard and Mathur (1985).

Individual infected kernels were dissected aseptically into six components including embryo, endosperm, palea, lemma, rachilla, sterile lemmas. These components were collected in small plastic bag, and then surface sterilized for 1 minute with 1% sodium hypochlorite solution (NaOCl). Each component of individual kernel was placed on 3 layers of moistened blotter in plastic petri dishes. The dishes were incubated at 24^{o}C ± 1^{o}C under 12h alternating cycles of near ultra violet (NUV) light and darkness. Each component was examined under a stereomicroscope for the growth of *Bipolaris oryzae* after 7 days of incubation with 4 replications (100 kernels/ replication).

2.2. Statistical analysis

Data was analyzed an analysis of variance (ANOVA) and mean comparison by SAS version 6.12 (SAS Institute Inc).

2.3. Results and Discussion

B. oryzae infection kernels with typical brown spot symptom on pericarp were collected. The components including embryo, endosperm, palea, lemma, rachilla, sterile lemmas were separated completely and incubated for 7 days. These components were examined under stereomicroscopes. The infection of *B. oryzae* was found all of these components at different levels. The rachilla was the highest at 82% of the infection and 79% on sterile lemmas (Table 1). Embryo and endosperm infection were lower than other sites, 14 % of infected was endosperm, 9 % embryo. Infection of lemma and palea was 61% and 55 %, respectively.

Table 1 Infection of *B. oryzae* on different components of rice kernel with brown spot symptom using blotter method after incubating at 24^{o}C ± 1 under 12h alternating cycles of near ultra violet (NUV) light and darkness for 12 h for 7days

Component	Infection (%)
Embryo	0.09c
Endosperm	0.14c
Lemma	0.61b
Palea	0.55b
Rachilla	0.82a
Sterile lemma	0.79a

CV (%) = 21

Mean followed by different letters in the row are significantly using Duncan's Multiple Range Test ($p \leq 0.05$).

The presence of *B. oryzae* on hull, pedicels (sterile lemma), pericarp, and seed coat was more than other parts of kernel similar to Nisikado and Nakayama (1943), Bernaux (1981), Suzuki (1985), Mew *et al*. (2002), and Khokon *et al*. (2005). There was no report about presenting of *B. oryzae* in embryo. However, according to Imam Fazli and Schroeder (1966) reported that the infection of the embryo might occur under favorable condition because they found the close association of hyphae with embryonic tissues. Consequently, this result showed that infection of rice kernel by *B. oryzae* had taken place through all the components of kernel and rachilla and sterile lemmas were mostly found infected.

Some of infected kernel with brown spot symptom on whole seed coat, embryo and endosperm also showed discoloration. Mycelia and conidia were produced only 3 to 4 days after incubation. On the lesion, conidiophores and conidia were produced directly.

3. Survival of *B. oryzae* in/ on kernel at different conditions

The survival of seedborne organisms and the survival of the host seed are intimately related. The conditions under which seeds are stored may affect the survival of the pathogen in / on the seed. Besides, the intrinsic nature of the organism and its location on or in the tissues of seeds also affect to the survival of organism. (Maude, 1996). Storage of rice kernel samples at different conditions to study how long *B. oryzae* in / on rice kernel can exist is an objective of this study.

3.1. Materials and methods

Kernel samples (Suphan Buri variety) were collected from the field with highly infection of *B. oryzae*, at Kasetsart University, Kampaengsaeng Campus, 2003, and stored at 10^{o}C. Sample was mixed thoroughly and testing the initial incidence of *B. oryzae* and then, divided to 2 sub samples. Each sub sample about 1,500g were contained in nylon bag and stored at 2 different conditions. One sample was stored in the low temperature incubator at 10^{o}C, the other was stored at room condition where temperature fluctuated between 18 – 41^{o}C, and relative humidity fluctuated every months of year (40 – 98 %), respectively. Kernel samples from two conditions were

sampled and checked every month to examine incidence of *B. oryzae* infected kernel by using blotter method after 7 days of incubation at 24^{o}C ± 1^{o}C under 12h alternating cycles of near ultra violet (NUV) light and darkness.

3.2. Statistical analysis

Data was analyzed an analysis of variance (ANOVA) and mean comparison by SAS version 6.12 (SAS Institute Inc). Statistics graphics were used by Sigma Plot 2000 program, version 6.0 (1986 – 2000 SPPS Inc).

3.3. Results and Discussion

The storage condition (at 10^{o}C) was similar with the condition used for kernel storage in the seed center and seed company. Likewise room condition was used similar to the condition which the farmers kept their seed for next seasons. The experiment was carried out from April 2004 to March 2005. The initial kernel infection of *B. oryzae* was 19. 25% and percentage of germination was 94.5 %. After 12 months of storage at 10^{o}C, the results showed that an infection of *B. oryzae* remained almost unchanged in this storage condition. After 12 months of storage, the infection of kernel (19.25%) still remained equally with initial infection (19.25%), and similarly resulted in the percentage of germination. Meanwhile, at the room condition, the results showed the reduction of percentage of infection and germination. After 3 months storage at room condition (from April to June, 2004), the infection was not significantly changed, but results showed that a significant decreased at the following months (From July, 2004 to March, 2005). Especially, the rapidly decreased infection of *B. oryzae* occurred between in June to August compared with initial infection. The following months, infection was still decreased but moderated or slightly. Totally, the decrease of the infection of *B. oryzae* for 12 months was from 19.25% to 9% (Figure 10). Besides, germination of rice kernel at room condition was also changed. The similar result was obtained as the infection when compared percentage of germination between months in the period of storage. There was also no significantly decreased after four months. However, the germination percentage gradually decreased after five months of storage (from July to

August, 2004) and the significantly decreased in February, and March 2005 (Figure 11). The different in germination of rice kernel between months of storage period might be caused by fluctuation of storage conditions in which both temperature and relative humidity were the major factors affected to germination.

It is evident from the results that survival of *B. oryzae* in/on rice kernel is depended on storage condition. Similar observations were reported by Nisikado *et al*. (1938), Page *at al*. (1947), Lam (1973). Recently, the effect of storage condition on the viability of *B. oryzae* was also studies by Mia *et al.* (1998). The results obtained different viability depending on the location of inoculum in which they found that for the external borne conidia could not survive longer than 135 days at 75.6%. At 26.8 to 66.8% RH, 18 – 42% of the conidia remained viable after 165 days of storage. In addition, under room condition only 1% of conidia survived by day 105 and none survived beyond. Meanwhile, the internal inoculum of pathogen in kernel remained 300 days at 25^{o}C, and under room condition remained unchanged.

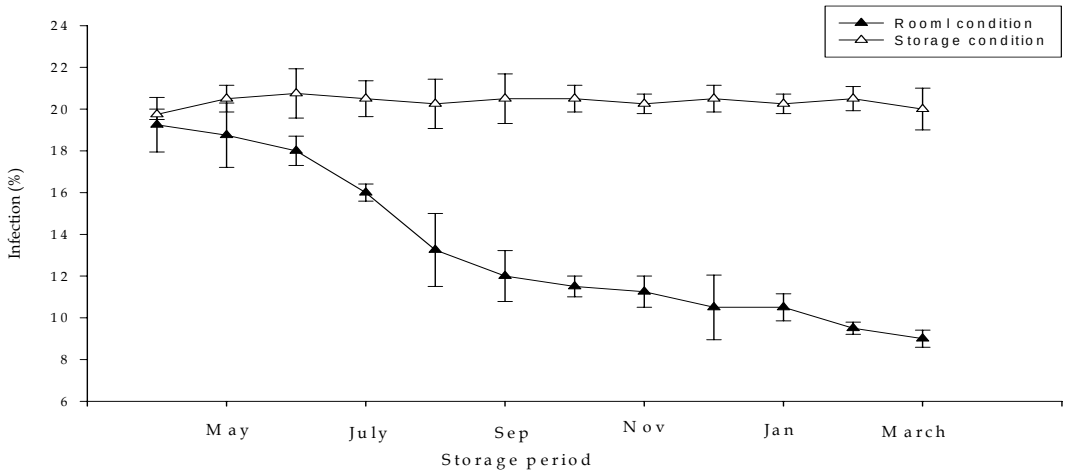


Figure 10 Survival of *B. oryzae* in /on the rice kernel at different storage conditions for 1 year.

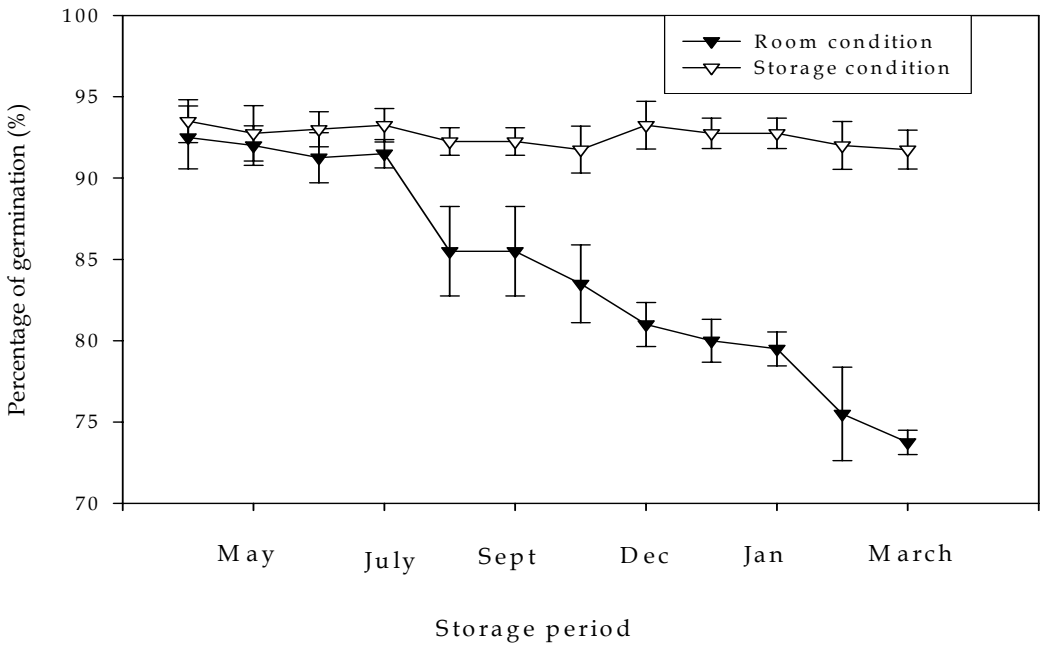


Figure 11 Effect of different storage conditions on the germination of rice kernel for 1 year.

4. Transmission of *Bipolaris oryzae* from infected kernel to seedling

Seed transmission refers to the passage of inoculum from an infected or infested kernel to a plant. Therefore, organisms may be seedborne but not seed transmission. The successful transmission from infected or infested seed to seedling depend on many factors including crop species, environment, and inoculum etc. Inoculum can be influenced by the amount of inoculum as well as type, virulence, and location of inoculum in seeds (Agarwal,1997). The objective of this experiment was to study transmission of *B. oryzae* from infected kernel to seedling and its correlation.

4.1. Materials and methods

4.1.1. Transmission of *Bipolaris oryzae* from infected kernel to seedling

Transmission of *Bipolaris oryzae* from infected kernel to seedling was studied under controlled environment in growth chamber and greenhouse using test tube agar, blotter method, and seedling symptom test respectively as described by Mathur

(2003). The infected kernel was obtained from the kernel lots collected from the same field. The infected kernel sample was tested by blotter method on the incidence of infected kernel before preceded to transmission study. The infected seedling was monitored for appearance of the symptom and disease progress development

- Blotter method: The infected kernels were surface sterilized with 1% sodium hypochlorite (NaOCl) and incubated on moistened blotter at $24^{o} \pm 1^{o}C$ in plastic tray with 100 pots (one kernel per pot was incubated) under 12 h alternating cycle of near ultraviolet (NUV) light and darkness for 7 – 14 days.

- Test tube agar: The infected kernels were surface sterilized with 1 % sodium hypochlorite (NaOCl) and then grown in test tube on 10 ml of water agar. The tubes were incubated at $24^{o}C \pm 1^{o}C$ under 12 h alternating cycle of near ultraviolet (NUV) light and darkness for 7 – 14 days. Infected seedlings were counted and confirmed by plating on 3 layers of moistened blotters in plastic petri dishes and checked for the growth of *Bipolaris oryzae* after 7 days of incubation under 12h alternating cycles of near ultra violet (NUV) light and darkness at $24^{o}C \pm 1^{o}C$.

- Seedling symptom test: The infected kernels were surface sterilized with 1 % sodium hypochlorite (NaOCl) and sown in the steriled sand tray using 400 kernels per sample(one kernel per pot was planted). The disease was also monitored after 21 days by washing to remove sand from the tray and examining under a stereomicroscope. The numbers of infected seedlings were confirmed and counted.

4.1.2. Relationship between infected kernel and seedling infection

Kernel sample of approximately 3 kilograms were collected in the field having a mean kernel infection of *B. oryzae* of 12 – 14% was used for this experiment. The kernel lot was uniformly mixed and divided in to 30 sub – sample. The samples were kept in plastic bags and stored at $10^{o}C$. Kernels from each bag were mixed thoroughly and divided to 2 parts for checking infection of *B. oryzae* by using blotter method and seedling symptom test. Four hundred was used for each test.

• Blotter method: Four hundred kernels were surface sterilized with 1 % sodium hypochlorite (NaOCl) and twenty-five kernels were placed on three layers of moistened blotters in plastic petri dishes. The dishes were incubated at 24^{o}C ± 1^{o}C under 12h alternating cycles of near ultra violet (NUV) light and darkness. The dishes were examined under a stereomicroscope for the growth of *B. oryzae* after 7 days of incubation.

• Seedling symptom test: Four hundred kernels were surface sterilized with 1 % sodium hypochlorite (NaOCl) and sown in the steriled sand tray (one kernel per pot was planted). The seedling with symptom and death were counted after 21 days. These infected seedling were confirmed by plating on 3 layers of moistened blotters in plastic petri dishes and checked for the growth of *B. oryzae* after 7 days of incubation under 12h alternating cycles of near ultra violet (NUV) light and darkness at 24^{o}C ± 1^{o}C.

4.2. Statistical analysis

Data was analyzed an analysis of variance (ANOVA) and mean comparison by SAS version 6.12 (SAS Institute Inc). Statistics graphics were used by Sigma Plot 2000 program, version 6.0 (1986 – 2000 SPPS Inc).

4.3. Results and Discussion

• Comparison of testing methods: Three methods, which were used in this experiment, were the most common method used in the health testing laboratories and the stations. These methods are simple and easy to practice. The experiment was conducted by using blotter, test tube agar and seedling symptom test to study transmission of *B. oryzae* from infected kernel to seedling. Infection kernel of *B. oryzae* using blotter and agar method obtained at 68% and 76.5% of colonization. Meanwhile, the seedling symptom test showed infection at 57% of seedling infection (Table 2).

• Test tube agar: Germination and seedling development was good. Moreover, the disease progress was monitored easily. The symptoms, mycelia and conidia were observed under stereomicroscope for all sites of seedling. However, mycelium of *B. oryzae* and other fungal could develop together. This obstructed the production of *B. oryzae* conidia.

• Blotter method: This method was good to follow disease progress from infected kernel to seedling of rice but it could not be used from germination to seedling stage with primary leave. The seedlings were weak and the blotter paper easily colonized by other fungi due to high moisture condition. With this method, the seedlings were observed directly under stereomicroscope and conidia were found on rootlets and coleoptiles of the infected seedlings.

• Seedling symptom test: The seedlings were well developed by this method. However, one limitation for monitoring disease progress was the symptoms on the roots of seedling. The seedling was taken off and cleaned to examine the symptom so the disease progress was not monitored consecutively on the same seedling.

Table 2 Transmission study of *B. oryzae* from infected kernel to seedling using blotter, test tube agar and sand method

Method	Infection (%)
Test tube agar	76.5 a
Blotter method	68 b
Seedling symptom test (Sand)	57 c

CV = 7.9%

Mean followed by different letters in the row are significantly using Duncan's Multiple Range Test (p = 0.05).

4.3.1. Seedling infection from infected kernel

Base on previous results and comparing advantages and disadvantages among methods, test tube agar method was used for studying of seed transmission of *B. oryzae*.

Infected kernel with typical symptom on pericarp was used for this experiment. One hundred infected kernels for one replication with four replications. The disease progress was examined after incubating at 24^{o}C ± 1^{o}C under 12h alternating cycles of near ultra violet light and darkness for 7, 14, and 21days.

Symptom on the coleoptiles and roots were observed. There was a significantly different on the occurrence of symptom on coleoptiles and root of seedling (table 3). The symptom as brownish to black necrotic spots on coleoptiles was occurred at 43.3 % and root at 11 %. Meanwhile, 18 % infection was observed on both coleoptiles and root. The infected seedlings with browning and etiolating of coleoptiles caused seedlings collapsed after 3 – 4 weeks. Some infected seedling, browning of coleoptile and death of seedling slowly progressed upward to primary leaves (22 %) (Table 3).

The roots of seedling infected by *B. oryzae* were discolored with a tinge of brown and became dark brown to black lesion during 10 - 14 days of incubation, and later, caused root distortion and rot. According to Suzuki (1930), Mundkur and Chattopahyay (1976), Ou (1985), and Rangaswami (1996) also observed the primary symptom occurred on the seedling was coleoptile and roots. These results supported that diseased kernel were an important source of primary infection to seedling. Coleoptiles and roots were primary site of infection and transmission of *B. oryzae* from infected kernel to seedling.

Table 3 Progression of *B. oryzae* infection from infected kernel to seedling and the appearance frequency of symptom on the seedling after incubating at 24°C ± 1°C under 12h alternating cycles of near ultra violet light and darkness for 7, 14, and 21days

Seedling	Part of seedling	Number of day of symptom appearance		
Infected seedling		7 days	10 – 14 days	18 – 21 days
	Coleoptile	34.5 ± 1.7 *	43.25 ± 2.17 *	43.25 ± 2.17*
	Roots	6.25 ± 0.85	11 ± 1.47	11 ± 1.47
	Coleoptile and roots	15 ± 1.29	18 ± 1.68	18 ± 1.68
	Primary leaf	N.D	0	22 ± 1.08
Ungerminated		N.D	18.25 ± 1.47	18.25 ± 1.47
Healthy seedling		N.D	9.5 ± 1.04	9.5 ± 1.04
Death seedling		N.D	N.D	8 ± 0.91
CV (%)		16.07	16.76	22.1

*Mean ± Std; N.D: Not Determined

4.3.2. Relationship between infected kernel and seedling infection

In this experiment, the samples were drawn from the kernel lot. Totally thirty sub-samples with different level infection were used for checking infection of *B. oryzae* in both blotter method and seedling symptom test. Relationship between infected kernel and seedling infection of each sample was determined using correlation analysis, and found positive and highly correlation ($r = 0.83$, $P < 0.0001$) (Figure 12).

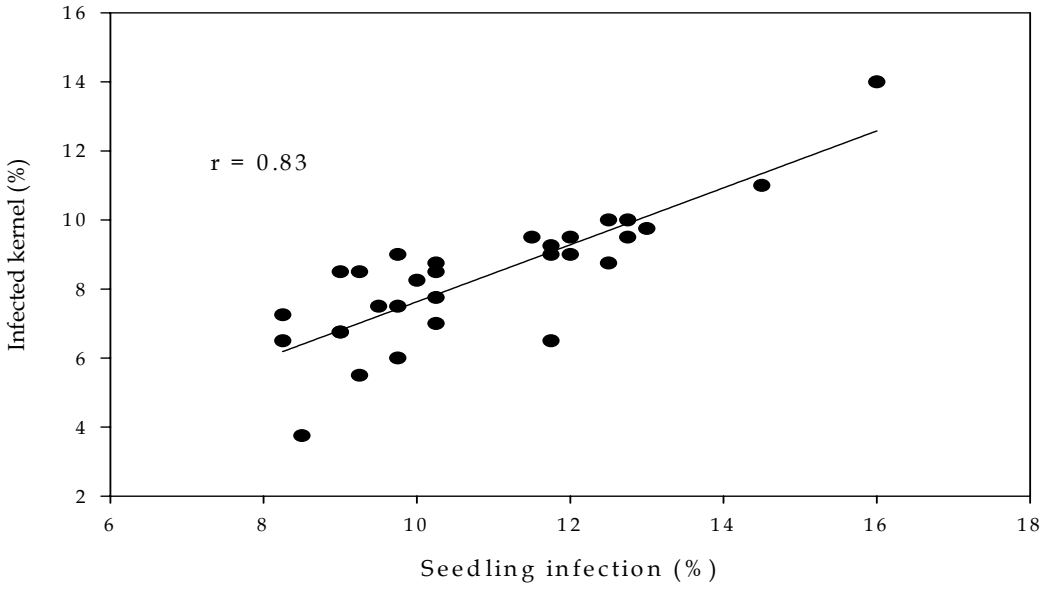


Figure 12 Relationship between infected kernel and seedling infection due to *B. oryzae* in/ on rice kernel by using blotter method and seedling symptom test (r = 0.83, P < 0.0001).

Similarly, the symptom appeared on coleoptiles and sometime on roots as the previous experiment. In some case, the seedlings were died because of heavy infection caused by the mycelia development. The incidence of infection in the blotter method obtained greater than that in the seedling test in almost all sample. This was probably because the conditions in the blotter method were favorable for growth of this fungus. In some case, the amount of inoculum which was carried with kernel was small, however, it could still have good growth because blotter is a method for detecting fungi. According to Rennie (1998) the successful transmission of pathogen to seedling depended not only on the amount and location of inoculum in/ on the kernel but also environmental conditions during germination and seedling establishment. However, the similar results were also obtained by Imolehin (1983), Kulik (1977), and Malavolta *et al*. (2002) showed significant correlation between incidence of *B. oryzae* and seed germination (r = -0.89), and seed incidence and death or infected seedling (r = 0.66). In addition, Mia *et al*. (2001) also reported that effect

of seedborne *B. oryzae* on seed germination and disease development in the field by mixing healthy seed lot with infected seed at different level, and results showed that reduction in the emergence of seedlings was corresponded with seed infection level.

5. Control of *B. oryzae* through Seed treatment

For some plant diseases, seed borne inoculum is a major source of infection. Therefore, reducing seed borne inoculum through seed treatment will be an effective disease control method (Rennie, 1998). According to Fourest *et al*.(1990); Grondeau *et al*.(1992); Detry (1993) seed treatment not only control the external and internal seedborne pathogens but also break the dormancy of seed (Zhang, 1990; Seshu and Dadlani, 1991). The seed treatment using dry heat and chemical were tested whether these treatments can control *B. oryzae* on/ in the kernel is an objective of these studies.

5.1. Materials and methods

5.1.1. Chemical seed treatment

• Effect of fungicides on rice kernel: Four fungicides were selected to use in this experiment including mancozeb (Dithane^R M-45), carbedazim (Bentox^R), mancozeb – carbedazim (Delsine Mx^R), and Carboxin – thiram (Vitavax^R 200). Each fungicide was tested on naturally infected kernel lot having a mean of kernel infection at 20 %. The fungicides were used at the rate of 2 g (a.i) per 1 kg seed. Kernel was treated with fungicides by using rotary seed treater for 30 minutes. The untreated control treatment was handled in the same way for all processes without fungicides. Kernels after treatment were placed on three layer moistened blotter in a petri dish with 25 kernels per dish. Four hundred kernels were used in each treatment. The preparation was then incubated at 24^{o}C ±1^{o}C under alternating period of 12 h near ultraviolet light (NUV) produced by Philips black light and 12 h dankness. After 10 – 14 days, infected kernels were recorded and identified. Germination of the kernel was also counted.

• Effect of fungicides on rice seedling: The same fungicides including mancozeb (Dithane^{R} M-45), carbedazim (Bentox^{R}), mancozeb – carbedazim (Delsine^{R} Mx), and Carboxin – thiram (Vitavax^{R} 200) also used in this experiment. The seed treatment was done as previous experiment using a kernel lot with known infection status (20 %). Four hundred kernels were used for each treatment and sown in plastic pots with moisture sterilized sand. They were incubated in growth chamber at 24 ±1^{o}C and alternating period of 12 h darkness and near ultraviolet light through the experiment period. The tap water was used to replenish moisture by adding everyday. Three weeks after sowing, the plant stand were recorded, all plants with symptom and expressed as percentage infection and number non germination kernel also were also recorded. After that, all plants were removed and washed in order to check root infection. Infection of *B. oryzae* was confirmed by isolation of each coleoptile and infected parts of seedling on 3 layer moistened blotters.

5.1.2. Dry heat treatment

Kernel samples used in this experiment were collected and stored at 10^{o}C, which known infection (10 %). All dry heat treatments were applied to kernel using fan assisted convection ovens. Ovens allowed stabilizing for 2 days prior to treatment of kernel. Temperatures were maintained within ± 2^{o}C. Kernel lots were placed in individual petri dishes and group within the oven to avoid variation. Four replications (100 kernels/ replication) were used for infection and germination. After treatment, kernel samples were placed in plastic bags and stored at 10^{o}C until all treatments within that experiment had been completed.

• Determination of kernel infection: Kernel samples were surface sterilized for 5 minutes in 1 % sodium hypochlorite and rinsed twice with sterile distilled water and after that 25 kernels were placed on 3 layer moistened blotters in petri dish. All petri dishes were incubated at 24^{o}C under 12 h light/ dark cycle. Each kernel was examined under stereomicroscope for sporulation of *B. oryzae* after 7 days incubation. The number of kernel infection and germination were counted according to Copeland and Mc Donald (2001).

5.2. Statistical analysis

Data was analyzed an analysis of variance (ANOVA) and mean comparison procedure by SAS version 6.12 (SAS Institute Inc). Statistics graphics were used by using Sigma Plot 2000 program, version 6.0 (1986 – 2000 SPPS Inc).

5.3. Results and Discussion

5.3.1. Effect of fungicides on inoculum of *B. oryzae* on rice kernel

Seed treatment with different fungicides significantly reduced the incidence of *B. oryzae* in/ on the infected kernel and seedling infection in both methods blotter and seedling symptom test (sand method) respectively (Table 4). Among these fungicides, combination carboxin - thiram (Vitavax^R 200) was the most effective in inhibition followed by Mancozeb- carbendazim (Delsine^R Mx), and mancozeb (Dithane^R M-45). All fungicides used as kernel treatment was effectively reduced infection of *B. oryzae* of seedling than untreated kernel. Carboxin –Thiram (Vitavax^R 200) inhibited completely infection of *B. oryzae* in blotter test, and reduced seedling symptom from 12 % to 2.3 %. Besides, carbendazim (Bentox^R) was not highly effective in both blotter and seedling symptom test method, only reduced infection from 22.3 % (blotter method) and 12 % (seedling symptom test) to 16.8 %, and 8 % respectively. Meanwhile, the adversary results obtained as carbendazim and mancozeb was combined the infection of *B. oryzae* reduced from 22.3 % (control) to 1.5 % in the blotter and 12 % to 2.8 % in the seedling test method. In these experiments, the seedling emerged from all fungicides treated kernels on both methods were equally or higher than untreated. Stand count of seedlings obtained from treated kernel were from 90 % to 95 %, and the lowest result was 90 % (untreated).

Table 4 Effect of fungicides seed treatment on incidence of *Bipolaris oryzae* on rice kernel and seedling using blotter and seedling symptom test after incubating at 24°C ± 1°C under 12h alternating cycles of near ultra violet light and darkness for 7days

Method	Blotter method		Seedling symptom test	
Fungicides	Germination (%)	Infection (%)	Germination (%)	Infection (%)
Control	89	22.3a	90	12a
Carbendazim (Bentox^R)	91	16.8b	90	8b
Mancozeb (Dithane^R M-45)	93	1.5c	94.3	3.5c
Man-Carb (Delsine^R Mx)	94	1.5c	91.3	2.8c
Carboxin-thiram (Vitavax^R 200)	93	0.0c	95	2.3c
CV(%)	29.72		32	

Mean followed by different letters in the column are significantly using Duncan's Multiple Range Test ($p < 0.05$).

The same results also obtained by Kauraw (1986); Buffa *et al.* (1995) when he used these fungicides to control fungi *B.oryzae*, *Fusarium spp*, *Alternaria padwickii*, and *Curvularia*. However, Kannaiyan *et al*. (1982) also revealed that carbendazim (bavistin) was effective against *Piricularia oryzae* but not *Cochliobolus miyabeanus*. An effective of these fungicides were also obtained by using seed treatment in reducing inoculum of *B. oryzae* in rice kernel such as Buffa *et al.* (1991) treated kernel with carbendazim - mancozeb, mancozeb, and carboxin - thiram for some rice cultivars to control of *Drechslera oryzae* resulted in better germination. According to Edgington *et al*. (1980), fungicides used in this experiment were systemic fungicides offered better control pathogens within the kernel as well as those on the surface. It was taken into the germinating kernel and protected the seedling during germination and development. Besides, these fungicides were combined with others also to inhibit some other fungi of rice kernel such as *Alternaria padwickii*, and *Curvularia* by using a combination of maneb and thiabendazole (Islam *et al*., 1992). *Alternaria alternata*, *Curvularia lunata*, *Sarocladium oryzae*, *Fusarium graminearum*, *F. moniliforme* by using combination carbendazim and mancozeb

(1:1), carbendazim and thiram (1:1) (Sachan *et al.*, 1994). In addition, according to Parisi *et al*. (2001), not only *P. oryzae, B. oryzae* but also *Microdochium oryzae* were eradicated by carbendazim - thiram or carboxin - thiram. Consequently, the combination among fungicides results more effective to control seedborne disease not only special pathogen but also other pathogens present on/ in the kernel.

5.3.2. Dry heat treatment

• Effect of different temperatures on *B. oryzae* in/ on rice kernel: In this experiment, the kernel sample with 10.75 % initial infection of *B. oryzae* and 10 % moisture content were exposed in the oven at varies temperature level 50, 60, 70, 80, and 90^{o}C (400 kernels for each treatment) to test whether the temperature level could be used to control the infection of *B. oryzae* in/ on rice kernel. The period of exposure for all these temperature was 24 hours. All samples were stored in refrigerator at 10^{o}C after exposing until all treatments of the experiment have completed. After that, the sample was checked for *B. oryzae* using blotter method. 7 days after incubation, rice kernels with infection of *B. oryzae* and germination were also counted.

The results showed that dry heat treatment at 50, 60^{o}C in 24 h exposure had no significant effect on reducing infection of *B. oryzae*. The infection was still 10.5 %, and 9.75 % when compare with control (10.75 %). Meanwhile, the significant reduced of infection obtained at 70, 80^{o}C, and nearly eradicated at 90^{o}C. The percentage of infection of *B. oryzae* had decreased from 10 % to 6.25 % at 70^{o}C, 5 % at 80^{o}C, and 0.75 % at 90^{o}C respectively. (Figure13). Similarly, the percentage germination after 24 h exposure of dry heat at 50, 60, and 70^{o}C was negligible effect (i.e. percentage germination at 50, 60, and 70^{o}C were 92.5; 93.25; and 91 % which was not significant different with control). However, the germination slightly effected at 80^{o}C, but still remained higher than 80 %. The percentage of germination was greatly affected at 90^{o}C, although the infection was nearly eradicated. The percentage of germination decreased from 93.25 % to 39.25 % (40 %). Besides, at higher temperature (80, 90^{o}C) germination was delayed. Rice kernel germinated later than

other temperatures one to two days. Consequently, exposing rice kernel at 70 and 80°C showed the most effective in reducing *B. oryzae* in/ on rice kernel.

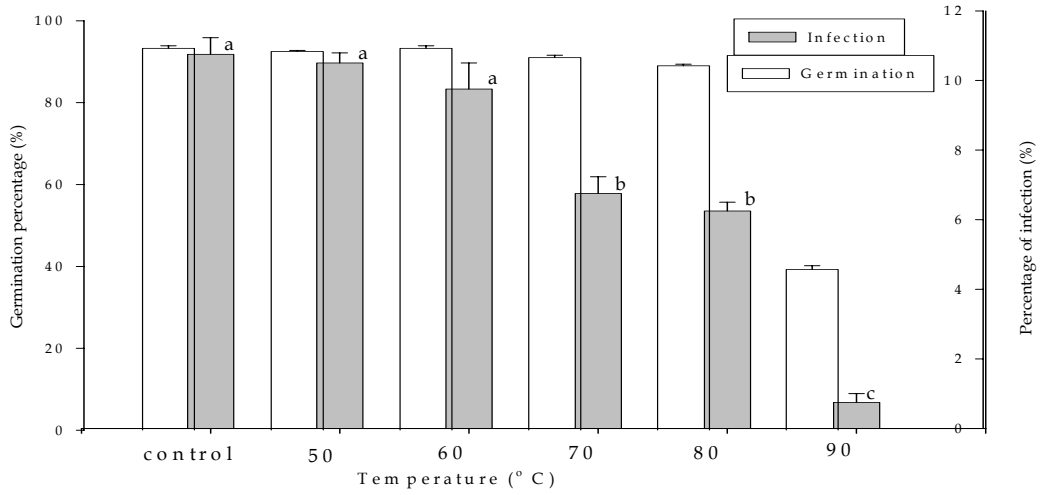


Figure 13 Effect of different temperatures (50, 60, 70, 80, 90°C) with 24 h of exposure on *B. oryzae* in/ on rice kernel.

• Effect of different periods of exposure to dry heat on the infection of *B. oryzae* in/ on rice kernel: This experiment was carried out based on the results of previous experiment that was to determine the period of exposure to heat at 70, and 80°C. Kernel samples with initial infection level of 10.75 % and 10 % moisture contain were exposed to dry heat at these temperatures for 24, 48, 72 and 96 h (400 kernels for each treatment). The results showed that the significantly difference in decreasing of *B. oryzae* infection obtained in all treatment times. The infection of *B. oryzae* rapidly decreased after 48, 72, and 96 h of exposure which the infection decreased to 6 % for 48 h, 4.75 % for 72 h, and 2.75 % for 96 h of 70°C exposure respectively. Likewise the infection of *B. oryzae* reduced to 5.75 %, 4.75% and 2.5% after exposing at 80°C for 48 h, 72 h, and nearly eradicated (0.75 %) at 96 h (Figure 14). However, kernel germination slightly effected at both 70 and 80°C. Especially, the percentage germination of rice kernel was greatly effected (decreased to 72 %) after exposing at 80°C for 96 h compared with control (Figure 15). In some previous studies, the infection of *B. oryzae* were also effectively reduced by using hot water at

50 – 55^{o}C for 10 -15 min (Thomas (1941); Suzuki (1930); Reyes (1939); Correa (1940); Chaudhuri (1948); Kulkarni (1980); Kulkarni (1980); and Krishnamurthy (2001).

Recently, there were some others studies which used dry heat treatment for eliminating the pathogen in/ on rice kernel. Zeigler *et al*. (1987); Detry (1993) used heat treatment against transmission of *Psedomonas fuscovaginae*, causal agent of bacterial sheath brown rot of rice at 65 ± 1^{o}C for 6 days and the result showed that the effects of disinfecting kernels in germplasm exchange program. In addition, Dadlani *et al.* (1990) also used dry heat treatment on rice and found that the infection of fungal decreased from 73.3 % (control) to 40 % after 5 and 6 days exposure to heat at 50^{o}C and 60^{o}C, and reduced to 38.3 % at 65^{o}C after 7 days of exposure respectively. Lee *et al*. (2001) also exposed other varieties of rice at different temperature levels (70, 75, 80, 85, and 90^{o}C) for period 24 h, found that the germination and seed vigor were no adverse effects at low temperature (70, 80^{o}C) but at higher temperature (85, 90^{o}C) were significantly reduced compared with control. Therefore, these results indicated that dry heat treatment reduced infection of *B. oryzae* in rice kernel at acceptable temperature level and period of exposure.

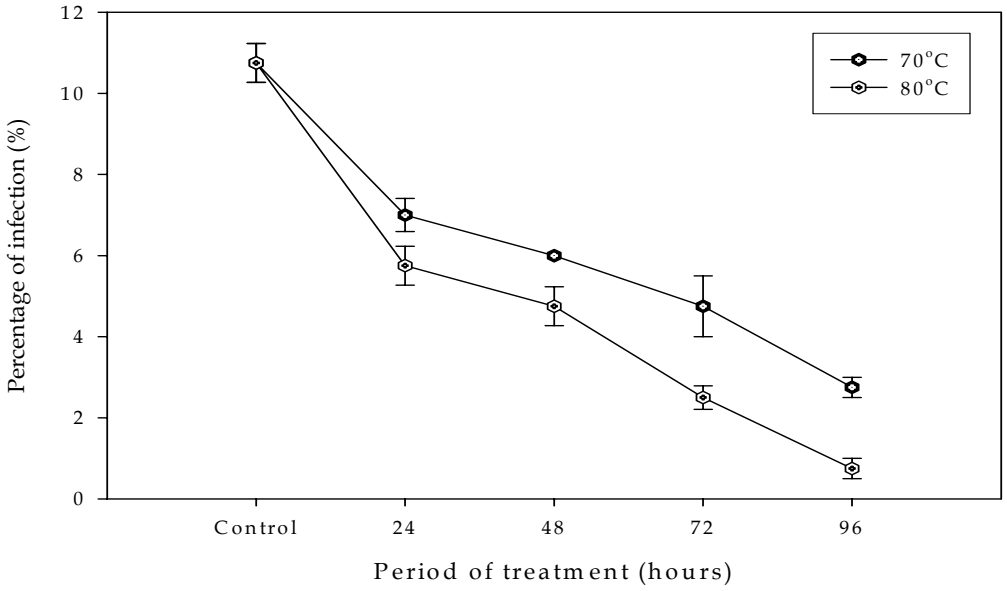


Figure 14 Effect of different periods (24, 48, 72, and 96 h) of exposure to heat on the infection of *B. oryzae* in/ on rice kernel.

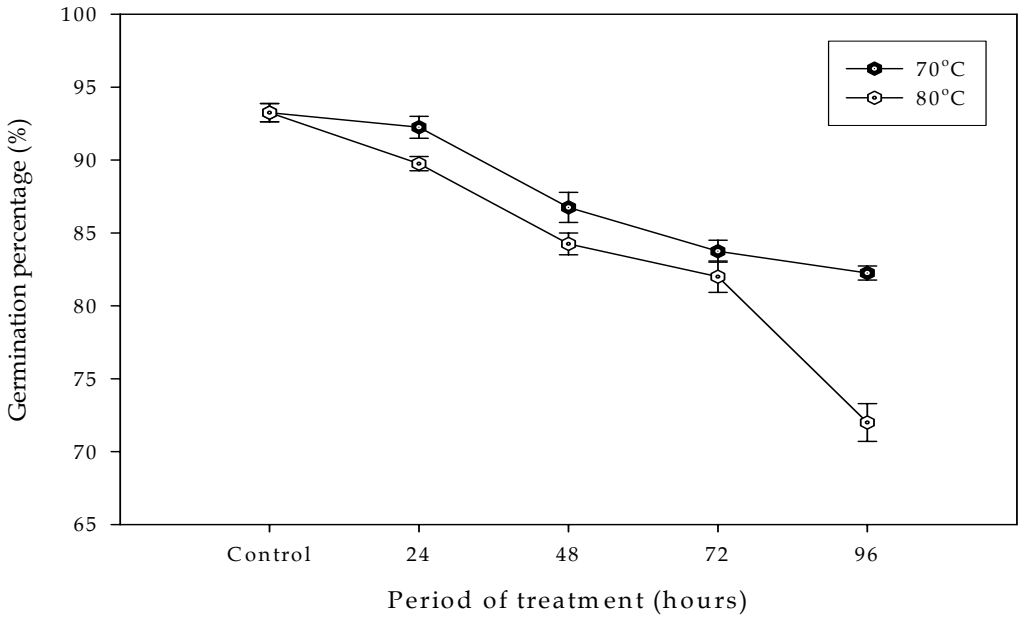


Figure 15 Effect of different period (24, 48, 72, and 96 h) of exposure to heat on germination of rice kernel.

GENERAL DISCUSSION

From these studies, *B. oryzae* found on all parts of rice kernel indicated that the fungus was colonized in and on the kernel. Therefore, the infection progress could be taken place during kernel setting. According to Watanabe *et al.* (1976) reported that the hyphal invasion of parenchymatous tissue through the inner epidermis of the hull at flowering. The germ tube passed through the space between the lemma and palea to reach the inner epidermis. These supported that seed coat, rachilla (pedicel), and sterile lemmas (empty glume) were observed highly frequency of the infection. The location of fungus in/ on or with kernel influenced to its longevity. The fungus was situated more deeply in the tissues of kernels protected from adverse microbiological and environment, should survive longer periods than others situated on superficiality (Maude, 1996). This was demonstrated by many pathogen on various kinds of kernels with different location having different survival periods, and *B. oryzae* was found in many studies. However, storage conditions in which temperature and relative humidity were the primary factors that affected the longevity of seedborne pathogen. There was no reduction of infection at 10^{o}C in 12 months showed that this storage condition was favorable for existence of *B. oryzae* in the kernel. In contrast, the significant decrease followed months at room condition (temperature 18 – 41^{o}C, RH: 50 – 95 %) because of improper condition of storage which was not only affected to the inoculum of infection in/ on kernel (Maude, 1996) but also affected to seed viability (Copeland and Mc Donald, 2001)

The seedling infection developed from infected kernel at high rate inferred that *B. oryzae* was transmitted by kernel (Neergaad, 1977), and the positive and highly correlation ($r = 0.83$, $P < 0.0001$) was measured by using correlation coefficient showed that the seedling infection was associated with infected kernel, therefore, infected kernel was the mean of transmission to seedling. After germination, the primary symptom of the seedling infection from infected kernel was on the coleoptile and roots. This indicated that the coleoptile and roots were exposed or more susceptible to infection. According to Chung and Lee (1983), Suzuki (1976) the fungus grown from the hilum through the pericarp to the extruded tip of the

scutellum to the coleoptile, and then to the primary leaf. Mycelia from the infected pericarp grown to the radicle and extended to the tip of the coleorhiza. Moreover, Suzuki *et al* (1977) reported that conidia produced on hulls or empty glumes reached the host tissues, such as coleoptile caused seedling infection. The study showed that seedborne *B. oryzae* caused reduced seedling emergence, seedling blight, and weakening of survived seedlings, suggested that at least in the nursery it could act as an important primary pathogen.

Difference in susceptibility of rice plants from flowering to dough stage indicated that flowering and milky were higher susceptible than others. At these stages, soft and tender young tissues offered less tolerant to fungal penetration. In the contrast, the mature tissues became more resistant (Imam Fazli *et al*., 1966). The progressive increased in both percentages of infected kernel and percentage of infected leaf area at three growth stages of rice plant: flowering, milky and dough obtained from the rice field was contrasted with inoculation in the greenhouse in which results obtained progressive decrease of pathogen. These suggested that infection could be taken place at the flowering stage and disease developed and spreaded continuously to the later stages once the conditions were favorable for development of pathogen (Percich *et al.*, 1997; Padmanabhan *et al*., 1954). Consequently, followed by losses in both yield and quality of seed at the harvested time.

The effective results in reduced infection of *B. oryzae* in/ on rice kernel were obtained in both chemical and dry heat treatment. Combination of seed treatment with two or more chemical could result more effective compared with only one chemical (Kauraw, 1986; Buffa *et al*., 1991; Islam *et al*., 1992). Besides, dry heat treatment also significantly reduced infection of *B. oryzae,* but in some case, the seed germinability was affected at high temperature and long exposing period (90^{o}C for 24 h, and 80^{o}C for 96 h). In addition, the samples which exposed at high temperature levels, the germinability were delayed 1-2 days. These could be caused by the kernel moisture content because of reduction during exposing time. According to Thomas *et al*. (2004) used longer periods of exposure (greater 4 days) or higher temperatures

(70^{o}C) should be reduced or eradicate infection in some cases but might reduce in seed viability. Using heat treatment should be concerned in both seed disinfection and seed viability for each seed lot. Further protection can combine heat treatment with other techniques or other measures. Consequently, it would be advisable to use seed treatment options as a component of integrated management measures.

CONCLUSION

It can be concluded from these studies that the relationship between severity of brown spot on flag leaf and incidence of infected kernel caused by *B. oryzae,* was highly significant correlated at 3 stages of rice plant: flowering ($r = 0.84$, $P < 0.0001$), milky ($r = 0.84$, $P < 0.0001$) and dough stage ($r = 0.80$, $P < 0.0001$). Disease progress of *B. oryzae* on the rice plant increased with development stages of the plant from flowering to dough stage. By artificial inoculation at three growth stages including flowering, milky and dough stage of rice plant, the most susceptible to an infection of *B. oryzae* was at flowering, and milky stage. Location of *B. oryzae* in/ on the infected rice kernel was observed in all components of kernel but mainly found on rachilla and sterile lemma. *B. oryzae* could survive in/ on rice kernel steadily under storage condition (10 ± 1^oC) but decreased slightly in each month at room condition (temperature fluctuated $18 – 41^oC$; RH: 40 – 98 %) after 12 months of storage. There was a relationship between infected kernel and seedling infection ($r = 0.83$, $P < 0.0001$). Coleoptiles and roots were primary infected sites on the seedling obtained from infected kernel. The fungicides seed treatment indicated that carboxin – thiram (Vitavax^{R}200) was found the most effective to control *B. oryzae* in/ on rice kernel followed by combination mancozeb - carbendazim (Delsine^R Mx), and mancozeb (Dithane^R M-45) whereas carbendazim (Bentox^R) was less effective against *B. oryzae* than others. Dry heat treatment also reduced *B. oryzae* infection in/ on rice kernel when exposed the kernels at 70^oC for 24, 48, 72, 96 h, and 80^oC for 24, 48, and 72 h. However, germination was affected at 90^oC for 24h of exposure and 80^oC for 96 h.

LITERATURE CITED

Agarwal, V.K. and J.B. Sinclair. 1997. **Principles of Seed Pathology**. 2nd Edition. CRC Lewis Publishers, Boca Rota, 539p.

Agarwal, P.C., C.N. Mortensen and S.B. Mathur. 1989. Seed-borne diseases and seed health testing of rice**. Phytopathological Papers**. 30. 106 pp.

Akai, S. 1965. *Helminthosporium* blight of rice plant with special reference to pathological physiology of the affected plants. **Annuals of The Phytopathological Society of Japan.** 31. 193-199.

Akai, S., J. Shinshiyama and R. Nishimura. 1965. Effect of spore density on the pathogenicity of *Helminthosporium oryzae* to rice leaves**. Ibid**.30: 166-168.

Aluko, M.O, 1975. Crop losses caused by the brown leaf spot disease of rice in Nigeria. **Plant Disease Reporter**. 59: 609 – 613.

Baba, I. 1958. Nutritional studies on the occurrence of *Helminthosporium* leaf spot and 'Akiochi' in rice plant. Bull. **Nat. Inst. Sci**. D7: 1-157.

Bedi, K.S. and H.S .Gill. 1960. Losses caused by the brown leaf spot disease of rice in the Punjab. **Indian Phytopathology**.13 (2): 161-164.

Bernaux, P. 1981. Evolution of the susceptibility of rice glumes to *Pyricularia oryzae* Cav, and *Drechslera oryzae* (Br. De Haan). **Agronomie.** 1(4): 261-264.

Buffa, G.N., G. Pelazza, G. Torazzo, L. Grassi and L. Tamborini. 1995. Seed treatment of rice for the control of Helminthosporiasis. **Informatore Agrario**. 51: 71-75.

Buffa, G.N., G. Pelazza, G. Torazzo, L. Grassi, L. Tamborini and R. Zecchinelli. 1991. Variations in the time of germination of rice seed samples treated for control of *Drechslera oryzae*. **Sementi Elette**. 37(2): 23-32.

Chandwani, G.H., M.S. Balakrishnan and S.Y. Padmanabhan. 1963. Helminthosporium disease of rice. V. A study of the spore population of *Helminthosporium oryzae* over rice fields. **J. Indian Bot. Soc.** 42(1): 1-14.

Chattopadhyay, A.K. and K.R. Samaddar. 1976. Effects of *Helminthosporium oryzae* infection and ophiobolin on cell membranes of host tissues. **Physiological Plant Pathology**. 8: 131-139.

Chattopadhyay, S.B. 1952. Assessment of infection of *Helminthosporium oryzae* Breda de Haan. **Proc. 39 th Indian Sci. Congr**. Part 3, p 35.

Chattopadhyay, S.B. and N.KChakrabarti. 1953. Occurrence in nature of an alternative host of *Helminthosporium oryzae* Breda de Haan**. Nature,** University of Kalyani. 172, 550.

Chattopadhyay, S. B. and Chakrabarti, N. K, 1954. Survival of *Helminthosporium oryzae* Breda de Haan in the field in nature. **Proc. 41st Indian Sci. Congr**. Part 3, 123p.

Chattopadhyay, S.B and C. Dasgupta. 1959. *Helminthosporium rostratum* Drechs. On rice in India. **Plant Disease Reporter**. 43: 1241-1244.

Chaudhuri, S.D. 1948. Appendix II. Annual report of the Economic Botanist, Assam, for the year 1945-1946**. Rep. Dep. Agric. Assam** 1945-1946: 85-170.

Chung H.S. and C.U. Lee. 1983. Detection and transmission of *Piricularia oryzae* in germination rice seed. **Seed Science and Technology**. 11: 625-637.

Copenald, L.O. and M.B. McDonald. 2001. **Principles of Seed Science and Technology**. Forth Edition. Kluwer Academic Publishers. London. 467 p.

Dadlani, M. and Seshu, D.V. 1990. Effect of wet and dry heat treatment on rice seed germination and seedling vigor. **International Rice Research Newsletter**. 15: 21-22.

Datnoff, L.E. and R.S. Lentini. 1994. Brown spot in Florida rice. **Florida Cooperation Extension Service**.University of Florida, 2p.

Dastur, J.F. 1942. Notes on some fungi isolated from 'black point' affected wheat seeds in the Central Provinces. **Indian Journal of Agricultural Science**.12: 731-742.

Detry, J.F. 1993. Seed dry-heat treatment against transmission of *Pseudomonas fuscovagivae*, causal agent of bacterial sheaths brown rot of rice (BSR). **International Rice Research Notes**. 18: 27-28.

Dhanapal, N., N.N. Prasad. 1979. Studies on the survival of the rice brown spot fungus *Helminthosporium oryzae* Breda de Hann in soil. **Madras Agricultural Journal**. 66(3): 165-169.

Dickson, J.G. 1956. **Diseases of Field Crops**. Second Edition. McGraw Hill Book Company, Inc. New York Toronto London, 429p.

Dreschsle, C. 1934. Phytopathological and taxonomic aspects of *Ophiobolus, Pyrenophora, Helminthosporium*, and a new genus, *Cochliobolus*. **Phytopathology**. 24: 953-985.

Duraiswamy, V.S. and V. Mariappan. 1983. Rice grain discoloration. **International Rice Research Newsletter**. 8(3): 9-10.

Feakin, S.D. 1970. **Pans Manual No 3 Pest Control in Rice**. The Ministry of Overseas Development, 270p.

Edgington, L.V., R.A. Martin, G.C. Bruin and I.M. Parsons. 1980. Systemic Fungicides: A Perspective after 10 Years. **Plant Disease**. 64(1): 19-23.

Fourest, E., L.D. Rehms, D.C. S., M. Bjarko and R.E. Lund. 1990. Eradication of *Xanthomonas campestris* pv. *translucent* from barley seed with dry heat treatments. **Seed Science and Technology**. 20: 816-818.

Fukatsu, R. and M. Kakizaki. 1955. Studies on the brown spot of rice plant. I. Sporulation on the diseased spot. **Annuals of the Phytopathological Society of Japan**. 19: 117-119.

Ganesan, T. and D. Lalithakumari. 1993. Longevity and infectivity of *Drechslera oryzae* conidia in soil. **Advances in Plant Sciences**. 6(1): 148-153.

Ganguly, N. 1946. Helminthosporium disease of paddy in Bengal. **Science and Culture**.12: 220-223.

Ghose, R.L.M., M.B. Ghatge and V. Subrahmanyan. 1960. **Rice in Indian**. New Delhi, Indian Council of Agricultural Research. 174 p.

Goto, I. 1958. Studies on the *Helminthosporium* leaf blight of rice plants. **Ibid**. 2: 237-388.

Grondeau, C., F. Ladonne, A. Fourmond, F. Poutier, and R. Samson. 1992. Attempt to eradicate *Pseudomonas syringae* pv. pisi from pea seeds with heat treatments. **Seed Science and Technology**. 20: 515-525.

Grummer, G. and S.K. Roy. 1966. Intervarietal mixture of rice and incidence of brown spot disease (*H. oryzae* Breda de Haan). **Nature,** University of Kalyani. 209: 1265-1267.

Hara, K. 1916. The sesame like leaf blight of rice plant. Nogyo Sekai 11(9). [Ja].

Hemmi, T. and K. Yokogi. 1928. Experimental studies on the pathogenicity of certain fungi on rice seedlings. **Mem, Coll. Agric**., **Kyoto imp. Univ**. 7: 1 – 22.

Hori, S. 1901. Leaf blight of rice plant. **Bulletin of the Central Agriculture Experiment Station**, Nishugahara, Tokyo 18: 67-84.

Imam Fazli, S.F. and H.W. Schroender.1966. Kernel infection of Bluebonnet 50 rice by *Helminthosporium oryzae*. **Phytopathology**. 56: 507-509.

Imam Fazli. and H.W. Schroender. 1966. Kernel Infection of Rice by *Helminthosporium oryzae* on Yield and Quality. **Phytopathology**. 56: 1003 – 1005.

Imolehin, E.D. 1983. Rice seedborne fungi and their effect on seed germination. **Plant Disease**. 67(12): 1334-1336.

Islam, M.K., A.J.M.M. Rahman and M.A.T. Mia. 2000. Significance of seed-borne fungal pathogens of rice with epidemiology. **Bangladesh Journal of Plant Pathology**. 16: 27-30.

Islam, M.K., M.A.T. Mia and M.A. Haque. 1992. Chemical control of seed-borne disease of rice. **Bangladesh Journal of Plant Pathology**. 8(1-2): 13-16.

Ito, S. 1932. Primary outbreak of the important diseases of the rice plant and common treatment for their control. **Rep. Hokkaido Agric. Exp. Sta**. 28: 204.

Ito, S. and K.Kuribayashi. 1927. Production of the ascigenous stage in culture of *Helminthosporium oryzae*. **Annuals of the Phytopathological Society of Japan**. 2: 1-8.

Kannaiyan, S. and T. Radhakrishnan. 1982. Effect of seed treatment with chemicals on the control of brown leaf spot and blast disease of rice in seedling stage. **Madras Agriculture Jounal**. 69(11): 769-770.

Katsura, K. 1937. On the relation of atmospheric humidity to the infection of the rice plant by *Ophyobolus miyabeanus* Ito and Kuribayashi and to the germination of its conidia. **Annuals of the Phytopathological Society of Japan**. 7: 105-124.

Kaur, P., S. Kaur and S.Y. Padmanabhan. 1979. Effect of manganese and iron on incidence of brown spot disease of rice. **Indian Phytopathology**. 32: 287-288.

Kauraw, L.P. 1986. Effect of fungicides on the germination, root/shoot growth and incidence of seed-borne pathogens in rice. **Indian Phytopathology**. 39(4): 609-610.

Kaur, P., S. Kaur and S.Y. Padmanabhan. 1984. Relationship of host nutrient status and brown spot disease expression in rice. **Indian Phytopathology**. 37: 156-158.

Kawada, A. 1954. Insects and Disease of Rice Plants in Japan. **Nat. Inst. Agric. Sci**. Tokyo. 33p.

Khatua, D.C., S. Maiti, C. Sen, S. Bandyopadhyau and D. Giri. 1978. Effect of fungicides on seedling health and brown spot of paddy. **Pesticides**. 12: 35-38.

Khonkon, M.A.R., M.B. Meah and G.A. Fakir. 2005. Inert matter with rice and wheat seeds is source of inoculum of plant pathogens. **Seed Science and Technology**. 33(1):127-140.

Klomp, A.O. 1977. Early senescence of rice and *Drechslera oryzae* in the Wageningen Polder, Surinam. **Agricultural Research Report**. Wegeningen No. 859.

Krishnamurthy, C.D., H.S. Shetty and S. Lokesh. 2001. Occurrence, transmission and remedial aspects of *Drechslera oryzae* in paddy (*Oryza sativa* L.). **Seed Research**. 63-70.

Kulkarni, S., R.K. Hegde and K. Ramakrishnan. 1980. Effect of hot water treatment of seeds on *Drechslera oryzae* (Breda de Haan) Subram. and Jain infection, vigor and yield of rice. **Mysore Journal of Agricuture Science**.14(3): 322-326.

Kulkarni, S., R.K. Hegde and K. Ramakrishnan. 1981. Epidemiology and control of brown leaf spot of rice caused by *Drechslera oryzae* (Breda de Haan) Subram. and Jain in Karnataka, II. Survival of *D. oryzae* in nature. **Current Research, University of Agricultural Sciences, Bangalore**. 10(4): 67.

Kulik, M. M. 1977. Seed germinability tests for predicting field emergence of rice seeds infected with *Helminthosporium oryzae* and *Trichoconis padwickii*. **Phytopathology**. 67(10): 1303-1304.

Kuribayashi, K. 1929. Overwintering and primary infection of *Ophiobolus miyabeanus*(*Helminthosporium oryzae*) with special reference to the controlling method. **Journal of Plant Protection**. 16: 25-36.

Lamaban P. S. and Z. Siddiqui. 2003. Effects of fly ash and *Helminthosporium oryzae* on growth and yield of three cultivars of rice. **Bioresource Technology**. 86: 73 – 78.

Lam, T.H. and L.B. Thrower. 1973. Viability and infectivity of hyphal fragments of *Helminthosporium oryzae* B. De Haan. **Phytopathologische Zeitschrift**.1: 42-45.

Lee, S.Y., Lee, J.H and Kwon T.O. 2002. Varietal differences in seed germination and seedling vigor of Korean rice varieties following dry heat treatment. **Seed Science and Technology**. 30: 311-321.

Long Ping Yuan. and Xi Qin Fu. 1995. **Technology of Hybrid Rice Production**. Food and Agriculture Organization of the United Nations. Rome. 84p.

Luh, S.B. 1991. **Rice Production**. Vol. I. 2nd Edition. Van Nostrand Reinhold. New York. 439p.

Malavolta, V.M.A, J.J.D. Parisi, H.M. Takada and M.C. Martins. 2002. Effect of different incidence levels of *Bipolaris oryzae* in rice seeds on physiological aspects, seed-seedling transmission and production. **Summa Phytopathologica**. 28(4): 336-340.

Mathur, S.B. and O. Kongsdal. 2003. **Common Laboratory Seed Health Testing Method for Detecting Fungi**, First Edition. International Seed Testing Association. Rome, 425p.

Marchetti, M.A. and H.D. Petersen. 1984. The role of *Bipolaris oryzae* in floral abortion and kernel discoloration in rice. **Plant Disease**. 68(4): 288-291.

Maude, R.B, 1996. **Seedborne Disease and Their Control. Principles and Practice**. CAB International. University Press. Cambridge, 280p.

Mew, T.W, and P. Gonzales. 2002. **A Handbook of Rice Seedborne Fungi**. Science Publishers. IRRI. Metro Manila. 83p.

Mia, M.A.T, and K.M. Safeeulla. 1998. Survival of seedborne inoculum of *Bipolaris oryzae*, the causal agent of brown spot disease of rice. **Seed Research**. 26: 78 – 82.

Mia, M.A.T, M. Rahman, D. Pearce and M. Holderness.2001. Effect of seed-borne *Bipolaris oryzae* on seed germination and disease development in the field. **Bangladesh Journal of Plant Pathology**. 17(1/2): 59-62.

Misra. A.P. and Y. Prasad. 1964. The nature of resistance of paddy to *Helminthosporium oryzae* Breda de Haan. **Indian Phytopthology**. 17(4): 287-295.

Mondal, A.H., M.A.T. Mia and A. Ali. 1998. Relationship between leaf spot and grain spot and planting value of spotted rice. **Seed Research**. 26(1): 73-77.

Mukherjee, A.K. and B.N. Bagchi. 1964. Control of secondary air borne infection of *Helminthosporium* disease paddy. **Rice News Teller**.12: 103-105.

Mundker, B.B. and S.B. Chattopahyay. 1967. **Fungi and Plant Disease**. Macmillan and Co. Limited, Calcutta, 348p.

Neergaard, P. 1977. **Seed Pathology**. Vol. I and II. The Macmillan Press Ltd., London and Basingstoke. 1187p.

Neergaard, P. and S. B. Mathur. 1985. **University Teaching of Seed Pathology**. Prasaranga, University of Mysore. 162p.

Nisikato, Y. 1923. Effect of temperature on the growth of *Helminthosporium oryzae* Bebra de Haan. **Annals of the Phytopathological Society of Japan**.1: 20-30.

Nisikato, Y., K. Hirata. and T. Higuti. 1938. Studies on temperature relations to the longevity of pure culture of various fungi pathogenic to plants. **Okayama Universitau.** 8: 107-124.

Nisikato, Y.. and C. Miyake. 1922. Studies on the Helminthosporiose of the rice plant. **Ibid**. 2: 133-194.

Nisikado, Y. and T. Nakayama. 1943. Notes on the pathological anatomy of rice grain as affected by *Helminthosporium oryzae*. **Ber. Ohara Inst. Landwirtsch. Forsch**. 9: 208 – 213.

Nyvall, R.F. and J.A. Percich. 1999. Development of fungal brown spot and spot blotch on cultivated wild rice in Minnesota. **Plant Disease**. 83(10): 936-938.

Nyvall, R.F., J.A. Percich, R.A. Porter. and J.R. Brantner. 1995. Comparison of fungal brown spot severity to incidence of seedborne *Bipolaris oryzae* and *B. sorokiniana* and infected floral sites on cultivated wild rice. **Plant Disease**. 79: 249 – 250.

Ocfemia, G.O. 1922. The sesame spot disease of rice.(Abs). **Phytopathology**, 12, p.34.

Ocfemia, G.O. 1923. The *Helminthosporium* disease of rice. **Phytopathology**. 13: 53.

Ocfemia, G.O. 1924. The relation of soil temperature to germination of certain Philippine upland and lowland varieties of rice and infection by the *Helminthosporium* disease. **Amer. J. Bot**. 6. 437- 460.

Ou, S.H. 1985. **Rice Diseases**. 2nd Edition. C.A.B Commonwealth Mycological Institute. Kew, London, 380p.

Padmanabhan, S.Y. 1953. Specialization in pathogenicity of *Helminthosporium oryzae*. **Proceedings of the 40th Indian Science Congress**, Part 4, Abs 18.

Padmanabhan, S.Y. and Ganguly, D. 1953. Testing of rice varieties resistant to helminthosporiose and blast. **Rice News Teller**.1(3): 107-110.

Padmanabhan, S.Y.1977. **Fungal Diseases of Rice in Indian**. Indian Council of Agricultural Research. New Delhi, 66p.

Padmanabhan, S.Y., K.R.R. Chowdry. and D. Ganguly. 1948. Nature and extent of damage caused by the disease. Helminthosporium disease of rice. **Indian Phytopathology**. 1: 34-47.

Padmanabhan, S.Y.and D. Ganguly. 1954. Relation between the age of rice plant and its susceptibility to *Helminthosporium* and blast diseases. **Proc. Indian Acad. Sci**. B 39 (2): 44 – 50.

Padmanabhan, S.Y., D. Ganguly. and G.H. Chandwani. 1966. *Helminthosporium* disease of rice. 8. Breeding resistant varieties, selection of resistant varieties of early duration from genetic stock. **Indian Phytopathology**. 19: 72-75.

Padmanabhan, S.Y., C.T. Abichandani, N.K. Chakrabartu. and S. Panaik. 1962. studies on *Helminthosporium* disease of rice. VI. Nutritional factors and disease expression. 2. Effect of potassium. **Proc. 49th Indian Sci. Congr**. Part 3. 148.

Page, R.M.., A.F. Sherf. and T.L. Morgan.1947. The effect of temperature ad relative humidity on the longevity of the conidia of *Helminthosporium oryzae*. **Mycologia**.39: 158-164.

Parisi, J.J.D., F.L. Leonel Junior. and V.M.A. Malavolta. 2001. Chemical control of seedborne fungi in rice seeds (*Oryza sativa* L.). **Summa Phytopathologica**. 27: 403-409.

Percich, J.A., R.F. Nyvall. and D.K. Malvick. 1997. Interaction of Temperature and Moisture on Infection of Wild Rice by *Bipolaris oryzae* in the Growth Chamber. **Plant Disease**. 81: 1193-1195.

Rangaswami, G. 1996. **Diseases of Crop Plants in Indian**. Third Edition. Prentical-Hall of Indian Private Limited, New Delhi, 498p.

Rangaswami, G. and M. Ramalingam. 1962. Survival of *Helminthosporium oryzae* in soil and its inhibition by *Bacillus mycoides*. **Phytopathology.** 52(4): 347-351.

Rath, G.C. 1974. Effect of seedborne infection of *Drechslera oryzae* on the grain weight germination and emergence of some high yielding varieties of rice. **Sci. Cult**. 40: 156-159.

Reyes, G.M. 1939. Rice Diseases and Methods of Control. **Philipp. J. Agric**. 10: 419-436.

Rennie, W. J. 1998. Seedborne Disease in **The Epidemiology of Plant Diseases**. Kluwer Academic, Dordrecht. 295-307. 460p.

Richardson, M.J. (1979). An Annotated List of Seedborne Pathogens: **ISTA Seed Health Testing Handbook Section**. 1.1: 186 – 192.

Sachan, I. P. and V.K. Agarwal. 1994. Efficacy of seed treatment of discoloured seeds of rice on seedborne inoculum, germination and seedling vigor. **Seed Research**. 22(1): 45-49.

Sato, K. 1964-1965. Studies on blight diseases of rice plant. Bulletin of the Institute of Agricultural Research, **Tohoku University** .15: 199-237.

Seshu, D.V. and M. Dadlani. 1991. Mechanism of seed dormancy in rice. **Seed Science Research**. 1: 187-194.

Sherf, A.F. and M.P. Robert, E.C. Tullis, and L.M. Thomas. 1947. Studies on factors affecting the infectivity of *Helminthosporium oryzae*. **Phytopathology**. 37: 181-290.

Shoemaker, R.A. 1955. Biology, cytology and taxonomy of *Cochliobolus sativus*. **Can. J. Bot**. 33 (6): 562-576.

Subramanian, C.V. and B.L. Jain. 1966. A revision of some graminicolous Helminthosporia. **Ibid**. 35: 352-355.

Suparyono, J.L.A.C. and I.P Oña. 2003. **Brown spot**. International Rice Research Institure, 4p.

Suzuki, H. 1930. Experimental studies on the possibility of primary infection of *Piricularia oryzae* and *Ophiobolus miyabeanus* internal of rice seeds. **Ann. Phytopath. Soc. Japan**, II: 245 – 275.

Suzuki. H. 1976. Seed transmission of rice blast disease and seedling blast. **Noyaku Shunju**. 32: 1-5.

Suzuki, H. 1985. Seed disinfection against blast, brown spot, and bakanae diseases. **Noyaku Shunju**. 50: 2 – 7.

Suzuki, H. and Y. Fujita. 1977. Seedborne disease of rice, blast and "Nae-imochi' Bull. Tohoku Natl. **Agric. Exp. Stn**. 55: 241-244.

Thomas, G.J. and K.G. Adcock. 2004. Exposure to dry heat reduces anthracnose infection of lupin seed. **Australasian Plant Pathology**. 33: 537-540.

Thomas, K.M. 1940. Detailed Administration Report of the Government Mycologist, Madras, for the year 1939-1940: Cited from Ou, S.H. 1985. **Rice Diseases**. 2nd Edition. C.A.B Commonwealth Mycological Institute. Kew, London, 380p.

Thomas, K.M. 1941. Detailed Admistration Report of the Government Mycologist, Madras, for the year. 1940-1941. 22pp: Cited from Ou, S.H. 1985. **Rice Diseases**. 2nd Edition. C.A.B Commonwealth Mycological Institute. Kew, London, 380p.

Tucker, C.M. 1923. Report of the Acting Pathologist. **Rep. Agric. Exp. Sta.** 16 – 18.

Valarini, P.J., S. Chiba. and C.C. Lasca. 1988. Efficiency of rice seed treatment with fungicides for controlling *Helminthosporium oryzae*. **Pequisa Agropecuaria Brasileira**. 23(1): 41-44.

Vidhyasekaran, P. 1980. The use of dichloromethane to incorporate fungicides in to rice seeds for control of *Drechslera oryzae*. **Seed Science and Technology**. 8(3): 357-362.

Watanabe, Y., O. Horino., H. Fujii, and A. Ezuka. 1976. Ecological studies on the panicle blight of rice plant caused by *Cochliobolus miyabeanus*. **Bull. Tokai-Kinki Natl. Agric. Exp. Stn.** 29: 80-105.

Wesely, E.G., K. Rajarathinam, M. Jayabalan, E.G. Ebenezar, and T. Sekar. 1996. Survival of two fungal pathogens associated with the grain discoloration of rice. **Journal of Ecobiology**. 8(1): 29-32.

Worawistitthumrong, A.S., Keoboonrueng. 1971. Brown spot, *Helminthosporium oryzae* Breda de Haan. **Rice diseases and Pests of Thailand**. Rice Protection Research Centre, Rice Department, Ministry of Agriculture, Thailand: 14-15.

Zad, S.J, and V. Khosravi. 2000. Investigation on important seed borne fungal diseases of dominant rice cultivars in Mazandaran (Iran). **Universiteit Gent**. 65(2): 587-592.

Zeigler, R.S., Ribiano, M. and Alvarez, E. 1987. Heat and chemical therapy to eradicated *Pseudomonas fuscovaginae* from rice seed. **International Rice Research Newsletter**. 12: 18-19.

Zhang, X.G. 1990. Physiochemical treatments to break dormancy in rice. **International Rice Research Newsletter**. 15.

APPENDICES

Appendix A

Appendix Table A 1 Result of analysis of variance of severity brown spot (%) at flowering, milky, and dough stage of rice plant in the field.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	13.2693469	6.63467345	121.70	0.0001
Error	42	2.28964121	0.05451527		

Appendix Table A 2 Result of analysis of variance of incidence infected kernel (%)at flowering, milky, and dough stage of rice plant in the field.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1558.4322843	779.216142	134.62	0.0001
Error	42	243.10678167	5.78825671		

Appendix Table A 3 Result of analysis of variance of incidence infected kernel (%) and severity brown spot (%) at flowering, milky, and dough stage of rice plant in the field (Mean and standard deviation of error).

Stages	Infected kernel		Infected leaf area	
	Mean	Std error	Mean	Std error
Flowering	12.3	1.91	0.36	0.054
Milky	15.12	2.29	0.55	0.07
Dough	26.01	2.9	1.599	0.395
CV (%)	27.8		13.4	

Appendix Table A 4 Result of analysis of variance of incidence infected kernel (%) at flowering, milky, and dough stage of rice plant by artificial inoculation in greenhouse.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	4281.66434190	1427.22144730	157.01	0.0001
Error	136	1236.20937051	9.08977478		

Appendix Table A 5 Result of analysis of variance of severity brown spot (%) at flowering, milky, and dough stage of rice plant by artificial inoculation in greenhouse.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	22.76686179	7.58895393	60.57	0.0001
Error	136	17.04059250	0.12529847		

Appendix Table A 6 Result of analysis of variance of incidence infected kernel (%) and severity brown spot (%) at flowering, milky, and dough stage of rice plant in the greenhouse(Mean and standard deviation of error).

Stages	Infected kernel		Infected leaf area	
	Mean	Std error	Mean	Std error
Flowering	16.66	0.71	1.21	0.059
Milky	11.81	0.43	1.22	0.064
Dough	6.62	0.33	0.76	0.056
Control	0.2	0.089	0.056	0.025
CV (%)	29.9		38.00	

Appendix Table A 7 Result of analysis of variance infection of *B. oryzae* on different components of rice kernel with brown spot symptom using blotter method after incubating at 24^{o}C ± 1 under 12h alternating cycles of near ultra violet (NUV) light and darkness for 12 h for 7 days.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	19968.70833333	3993.74166667	34.23	0.0001
Error	18	2100.25000000	116.68055656		

Appendix Table A 8 Result of analysis of variance survival of *B. oryzae* in /on the rice kernel at different storage conditions.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Room condition					
Model	11	608.91666667	55.35606061	6.21	0.0001
Error	36	321.00000000	8.91666667		
Storage condition					
Model	11	8.06250000	0.73295455	0.14	0.9922
Error	36	183.75000000	5.104166667		

Appendix Table A 9 Result of analysis of variance effect of different storage conditions on the infection *B. oryzae* in/ on rice kernel (Means and Standard deviation of error).

Month	Room condition		Storage condition	
	Mean	Std error	Mean	Std error
April 2004	19.25a	1.31	19.75	0.25
May2004	18.75a	1.54	20.50	0.64
June 2004	18.00a	0.70	20.75	1.18
July 2004	16.00ab	0.41	20.50	0.86
August 2004	13.25bc	1.75	20.25	1.18
September 2004	12.00bc	1.22	20.50	1.19
October 2004	11.50bc	0.50	20.50	0.64
November 2004	11.25c	0.75	20.25	0.47
December 2004	10.50c	1.55	20.50	0.64
January 2005	10.50c	0.65	20.25	0.47
February 2005	9.50c	0.29	20.50	0.58
March 2005	9.00c	0.41	20.00	1.00
CV (%)	22.46		11	

Appendix Table A 10 Result of analysis of variance effect of different storage conditions on the germination of rice kernel.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Room condition					
Model	11	1869.16666667	169.92424242	11.91	0.0001
Error	36	513.50000000	14.25388889		
Storage condition					
Model	11	17.66666667	1.60506061	0.30	0.9813
Error	36	192.00000000	5.33333333		

Appendix Table A 11 Result of analysis of variance effect of different storage conditions on the germination of rice kernel (Means and Standard deviation of error).

Month	Room condition		Storage condition	
	Mean	Std error	Mean	Std error
April 2004	92.50	1.93	93.5	1.32
May2004	92.00	1.22	92.75	1.70
June 2004	91.25	1.54	93.00	1.08
July 2004	91.50	0.87	93.25	1.03
August 2004	85.50	2.75	92.25	0.85
September 2004	85.50	2.75	92.25	0.85
October 2004	83.50	2.39	91.75	1.44
November 2004	81.00	1.35	93.25	1.47
December 2004	80.00	1.32	92.75	0.94
January 2005	79.50	1.04	92.75	0.94
February 2005	75.5	2.87	92	1.47
March 2005	73.75	0.75	91.75	1.19
CV (%)	4.47		2.47	

Appendix Table A 12 Result of analysis of variance of different methods used for transmission study of *B. oryzae* from infected kernel to seedling including blotter, test tube agar and sand method.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.07646667	0.03823333	13.60	0.001
Error	9	0.02530000	0.00281111		

Appendix Table A 13 Result of analysis of variance the progression of *B. oryzae* infection from infected kernel to seedling and the appearance frequency of symptom on of seedling after 7 days incubation.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1673.16666667	836.58333333	130.38	0.0001
Error	9	57.75000000	6.41666667		

Appendix Table A 14 Result of analysis of variance the progression of *B. oryzae* infection from infected kernel to seedling and the appearance frequency of symptom on of seedling after 14 days incubation.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2959.20000000	739.80000000	66.15	0.001
Error	15	167.75000000	11.18333333		

Appendix Table A 15 Result of analysis of variance the progression of *B. oryzae* infection from infected kernel to seedling and the appearance frequency of symptom on of seedling after 21 days incubation.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	3491.21428571	581.86904762	52.27	0.0001
Error	21	233.75000000	11.13095238		

Appendix Table A 16 Result of analysis of variance effect of fungicides seed treatment on incidence of *Bipolaris oryzae* in/ on rice kernel and seedling using blotter and seedling test

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1709.300000	427.325000	68.55	0.0001
Error	15	93.500000	6.233333		

Appendix Table A 17 Result of analysis of variance effect of dry heat treatment at different exposure temperatures on the infection of *B. oryzae* in/ on rice kernel.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	289.20833333	57.84166667	70.59	0.0001
Error	18	14.75000000	0.81944444		

Appendix Table A 18 Result of analysis of variance effect of dry heat treatment at different exposure temperatures on the germination of rice kernel.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	9257.70833333	1851.54166667	1139.41	0.0001
Error	18	29.25000000	1.62500000		

Appendix Table A 19 Result of analysis of variance effect of dry heat treatment at different exposure temperatures on the germination of rice kernel and infection of *B. oryzae* in/ on rice kernel (Means and Standard deviation of error).

Temperature (°C)	Infection (%)		Germination (%)	
	Mean	Std error	Mean	Std error
50	10.5a	0.29	92.5	0.25
60	9.75a	0.75	93.25	0.62
70	6.75a	0.48	91.00	0.58
80	6.25b	0.25	89.00	0.41
90	0.75b	0.25	39.25	0.95
Control	10.75a	0.48	93.25	0.63
CV (%)	12		1.53	

Appendix Table A 20 Result of analysis of variance effect of dry heat treatment at different exposure periods to infection of *B. oryzae* in/ on rice kernel.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	141.50000000	35.37500000	43.32	0.0001
Error	15	12.25000000	0.81666667		

Appendix Table A 21 Result of analysis of variance effect of dry heat treatment at different exposure periods on the germination of rice kernel.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	390.80000000	97.70000000	43.42	0.0001
Error	15	33.75000000	2.25000000		

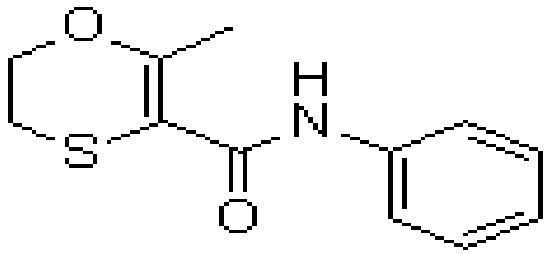
Appendix Table A 22 Result of analysis of variance effect of dry heat treatment at different temperatures and exposure periods on the germination of rice kernel and infection of *B. oryzae* on/ in rice kernel (Means and Standard deviation of error)

Temperature	70 ± 2°C				80 ± 2°C			
Period (hour)	Infection (%)		Germination (%)		Infection (%)		Germination (%)	
	Mean	Std error	Mean	Std error	Mean	Std error	Mean	Std error
24	7.0	0.41	92.25	0.75	5.75	0.48	89.75	0.48
48	6.0	0	86.75	1.03	4.75	0.48	84.25	0.75
72	4.75	0.75	83.75	0.75	2.5	0.29	82.00	1.08
96	2.75	0.25	82.25	0.48	0.75	0.25	72.00	1.29
Control	10.75	0.48	93.25	0.63	10.75	0.48	93.25	0.63
CV (%)	14.45		1.71		16.66		2.13	

Appendix B

1. Chemical structure and properties of carboxin

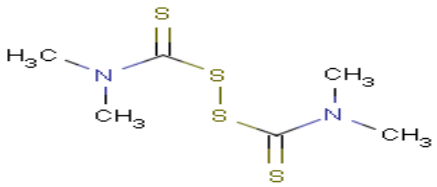
- Molecular Structure:



- Empirical formula: $C_{12}H_{13}NO_2S$
- Synonyms: Vitavax; 5,6-dihydro-2-methyl-1,4-oxathi-ine-3-carboxanilide; 5,6-dihydro-2- methyl-N-phenyl-1,4-oxathiin-3-carboxamide.

2. Chemical structure and physical properties of thiram

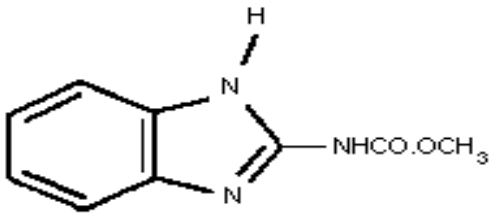
- Molecular Structure



- Chemical name: Tetramethylthiuram disulfide
- Empirical formula: $C_6H_{12}N_2S_4$
- Synonyms: bis(Dimethylthiocarbamoyl) disulfide; Tetramethylthiuram disulfide; Thiram

3. Chemical structure and properties of carbendazim

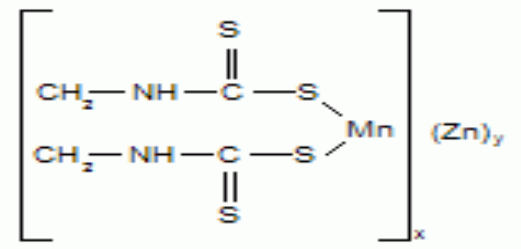
- Chemical structure:



- Empirical formula: $C_9H_9N_3O_2$
- Common name: Carbendazim (BSI, ISO)
- CAS chemical name: Methyl (1H-benzimidazol-2-yl)carbamate
- IUPAC chemical name: Methyl benzimidazole-2-ylcarbamate
- Synonyms: carbendazol (ZMAF), methyl-2-benzimidazole carbamate (MBC, MCB, BCM, BMC)

4. Chemical structure and properties of mancozeb

- Structural Formula:



- Empirical Formula: $(C_4H_6MnN_2S_4)x(Zn)y$
- Chemical Name: mangaese ethylenebis (dithocarbamate) (polymeric) coplex with zinc salt

Appendix C

Media used in the thesis research

Type	Ingredient	Quantity(gram)
Potato Dextrose Agar(PDA)	Potato	200
	Dextrose	20
	Agar	12
	Distilled Water	1000 ml
Potato Dextrose Agar + Rice Straw Extraction	Potato	200
	Dextrose	20
	Agar	12
	Rice Straw Extraction	5
	Distilled Water	1000 ml
Water Agar	Agar	20
	Distilled Water	1000 ml